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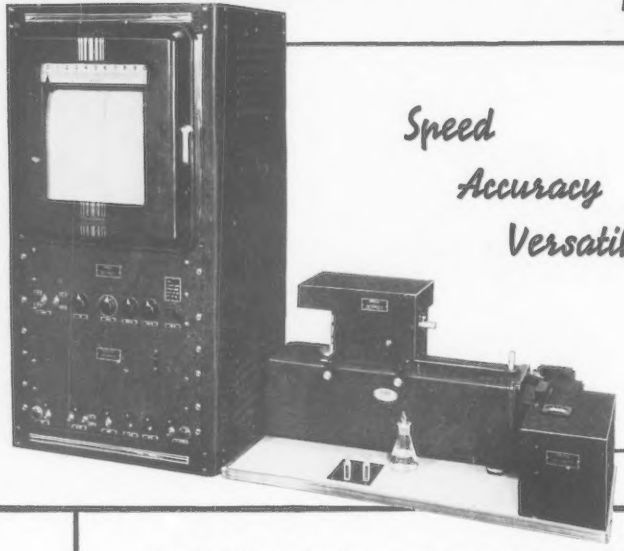

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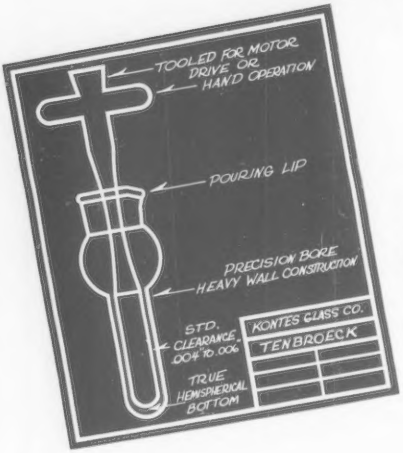

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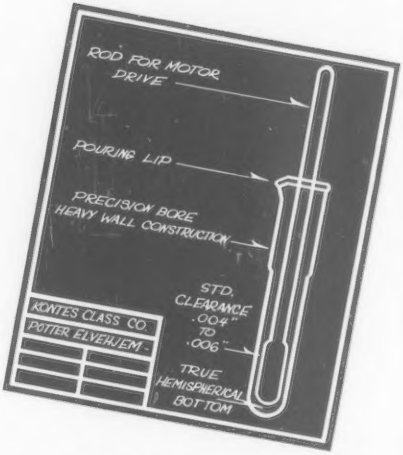

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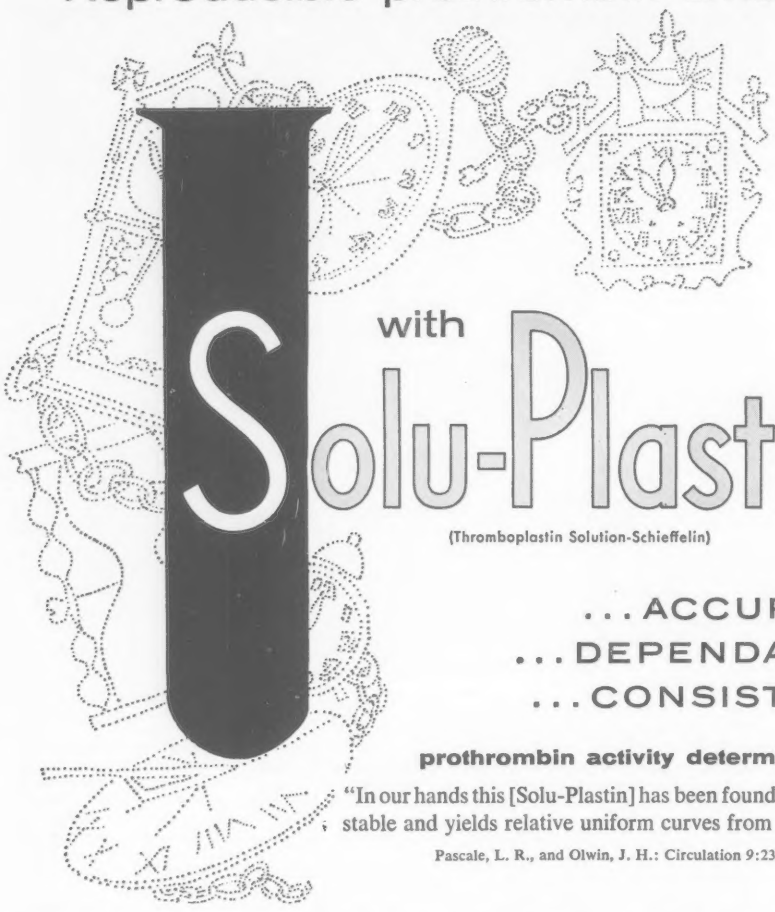

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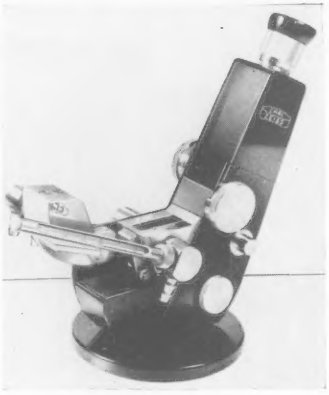

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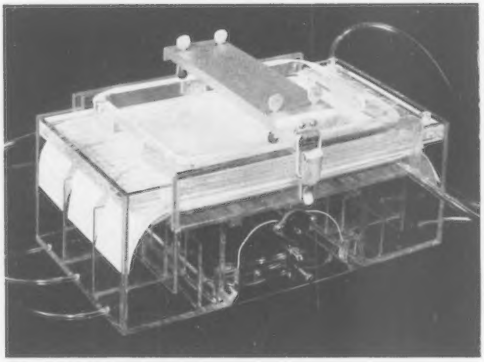

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1. Recent Progress in Hormone Research, New York, Academic Press, Inc., 1954, vol. 9, pp. 44, 411, 440. 2. Yearbook of Pathology and Clinical Pathology, Chicago, The Year Book Publishers, Inc., 1953-1954, p. 262. 3. J. Clin. Investigation *32*:246 (Aug.) 1953. Ibid. *33*:136 (Feb.) 1954. 4. Proc. Soc. Exper. Biol. & Med. *86*:266 (June) 1954. 5. Surg., Gynec. & Obst. *97*:709 (Dec.) 1953.

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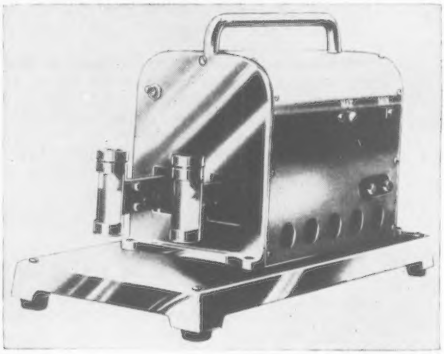

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Ref: J. Biol. Chem., *195*, 239 (1952)

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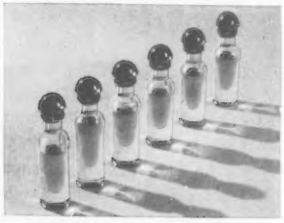

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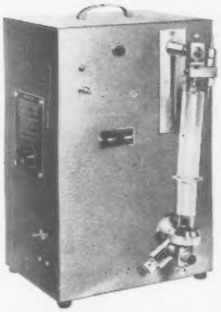

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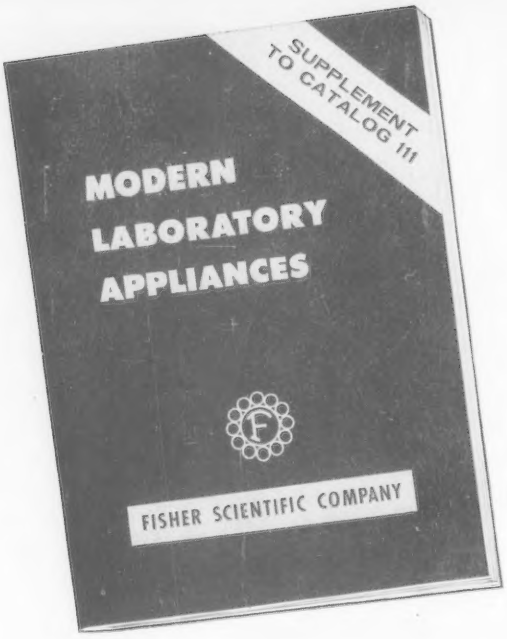

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CLINICAL CHEMISTRY

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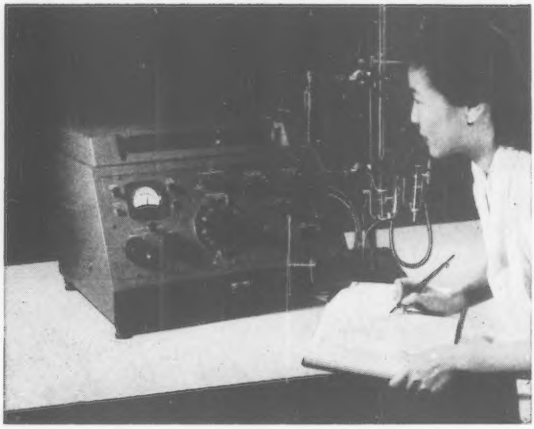
Chemical Evaluation of the Functions of the Liver (*Revised*)
John G. Reinhold 351

The Clinical Chemist 422

The American Association of Clinical Chemists: Program of the Annual Meeting 427

Index 429

TISSUE CHLORIDES?



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Chemical Evaluation of the Functions of the Liver

(Revised)

John G. Reinhold

STRUCTURE OF THE LIVER

The liver of a healthy adult weights 1650 Gm. on the average and it is by far the largest gland in the body. It consists of lobes which vary in size and differ to some extent in the sources of their afferent blood supplies. The liver is encased in a membrane of connective tissue. This extends into the liver at the transverse fissure of the undersurface (porta) in the form of a tree with innumerable branches. This framework of connective tissue supports the parenchymal cells, blood vessels, and bile ducts. The portal vein, hepatic artery, hepatic vein, and bile ducts enter the liver through the porta also, and undergo equally extensive branching. In its finer structure the liver consists of lobules 1–2 mm. in diameter. The lobules consist of numerous tubular secretory units, each formed by parenchymal cells surrounding a bile canaliculus.

The recent investigations of Elias (1949, 1953) have clarified structural relationships of the hepatic lobule to the vascular and biliary systems. Elias finds that the normal adult liver consists of many plates of epithelial liver cells one cell in thickness. The thickness of the plates may increase to 2 or more cells during regeneration of the liver. In lower animals also thicker plates are observed. The plates meet at varying angles to form a labyrinth of communicating cavities of irregular shape. Elias and Sokol (1953) state that the arrangement of the plates depends upon blood pressure and flow within the lobules.

From the William Pepper Laboratory of Clinical Medicine, Hospital of the University of Pennsylvania, Philadelphia, Pa.

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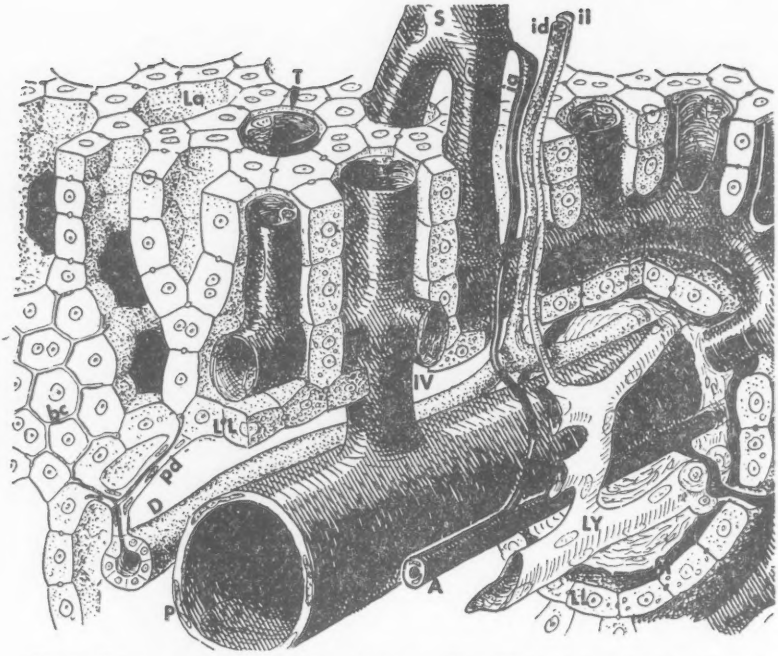

Fig. 1. Sterogram of the normal liver (drawn by Hans Elias). *A*, hepatic artery branch; *bc*, bile capillary; *D*, interlobular bile duct; *ia*, intralobular arteriole; *id*, intralobular ductule (cholangiole); *il*, intralobular lymph vessel; *IV*, inlet venule; *K*, Kupffer cell; *La*, labyrinth; *LL*, limiting plate; *LY*, portal lymph vessel; *P*, portal vein branch; *pd*, periportal ductule; *S*, sinusoid; *T*, tissue space of Disse. (From Popper, H., *Am. J. Med.* 76, 99, 1954. Reprinted with permission of the author and the publisher.)

The bile capillaries, according to Popper (1954), lie in grooves between the epithelial cells and may represent a special adaptation of the cell membranes of the latter. These join to form a "chicken-wire meshwork within the liver cell plates," and in turn continue into small bile capillaries (cholangioles). The cholangioles are lined with epithelial cells differing structurally and cytochemically from the liver cells (Fig. 1). Cholangioles arise from the liver cells embryologically and may proliferate excessively as a result of disease or injury of the liver, and especially during regeneration of the liver. This proliferation and secreting activity associated with it may be reflected in characteristic chemical changes in the blood.

Blood from the portal vein fills the sinusoids in the labyrinthine cavities between the plates of liver cells. This blood is enriched by arterial

blood from the hepatic artery entering the sinusoids by way of intralobular arterioles. The latter are equipped with sphincters capable of regulating over wide limits the flow of arterial blood into the sinusoids.

Blood flows through the sinusoids in a direction opposite to that of the flow of bile (Fig. 2). It appears that blood entering the portal vein from the superior mesenteric and the splenic veins maintains discrete lines of flow with relatively little mixing. India ink injected into the former is deposited only in the right lobe of the liver; that injected into the splenic vein is confined to the left lobe [Sérégé, H. J., (1901) cited by Wakim, (1954)]. As a result, the right lobe of the liver receives blood well supplied with nutrients predominantly from the small intestine; whereas the left lobe of the liver receives its blood mainly from the spleen and large intestine. One would expect therefore that the left lobe would be more vulnerable to noxious substances formed in the large intestine by action of micro-organisms.

Attached to the lining of the sinusoids are Kupffer's cells, large star-shaped or pyramidal cells, which are a part of the reticuloendothelial system. As such, they are phagocytic and contribute to the destruction of erythrocytes, hemoglobin breakdown, and bile pigment production. Some estimates place the number of reticuloendothelial cells in the liver at one third of the total number of cells. They may contribute to antibody production, and some workers believe them to be an important source of γ globulins. Cholesterol given to rats by the oral route is concentrated by the reticuloendothelial cells of the liver to a much greater extent than by the parenchymal cells (St. George and Friedman, 1955).

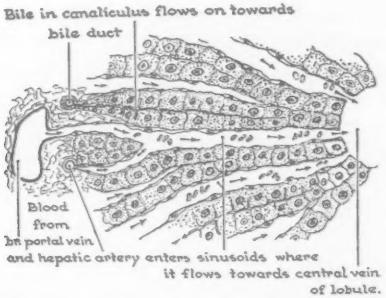
Bile in canaliculus flows on towards bile duct
Blood from portal vein and hepatic artery enters sinusoids where it flows towards central vein of lobule.

Fig. 2. Blood from the portal vein and the hepatic artery (left) flows into sinusoids, lined by reticuloendothelium, that lie between liver cords and empties into the central vein (right). Bile travels in the opposite direction in canaliculi to empty into bile ducts in portal areas. (From Ham, A. W., *Histology* (ed. 2), Philadelphia, Lippincott, 1953.)

The total volume of blood flowing through the liver was found by Bradley *et al.* (1945) to average 1500 ml. per minute, the range being 1085 to 1845 ml. per minute. Of this, about 12–44 per cent is arterial. However, the arterial blood contributes up to 60 per cent of the oxygen supply.

DISEASES OF THE LIVER AND BILIARY TRACT

Disturbances of metabolism occurring in liver disease may be sufficiently severe to jeopardize survival. They may be the result of (1) failure of the parenchymal cells to carry out vital functions because of infections or noxious agents; (2) decreased mass of functioning parenchymal cells resulting from disease; (3) decreased availability of blood to the liver cells because of (a) distortion of the liver architecture by scar tissue, (b) prolonged disturbance of the mechanisms regulating flow of blood in the liver with consequent shunting of blood around the liver or through it, (c) extrahepatic interference with blood supply, (4) impaired nutrition, (5) reaction of other organs to liver damage, e.g. brain, kidney, pancreas, adrenal, gonads, spleen, (6) injudicious therapy.

In a recent article of outstanding interest, Popper (1954) has revised and amplified many concepts of the morphologic changes occurring in liver disease. Among the changes he describes are:

1. Liver cell degeneration characterized by
   (a) loss of basophilic cytoplasmic nucleic acids, progressing at times to coagulation of the cytoplasm, or
   (b) marked swelling and rarified cytoplasm, found in hypoxia and carbon tetrachloride poisoning.
2. Atrophy, a thinning of the liver cell plates, associated with debilitating disease, senility, starvation, compression caused by biliary obstruction, or deposition of amyloid or of excessive amounts of glycogen.
3. Necrosis, an advanced degree of degeneration, characterized by loss of nuclear staining and ultimate disappearance of the cell. It may be
   (a) localized, with death of one or more cells and appearance of scavenger cells such as segmented leukocytes and lymphocytes.
   (b) central, involving all cells of the central zone of the lobule. This type occurs in severe hypoxia due to congestion or shock. It has been observed also in thyrotoxicosis and heart failure (Myers *et al.*, 1950).
   (c) periportal, involving the periphery of the lobule.
4. Regeneration, in which cells grow rapidly into areas destroyed by necrosis. Regeneration also may be stimulated in parts of the liver remote from the lesion. Proliferation of bile ducts is now regarded as evidence of liver cell regeneration.

5. Fatty metamorphosis, usually starting in the center of a lobule occurring as a result of
   (a) toxic injury
   (b) hypoxia
   (c) hormonal imbalance
   (d) nutritional deficiency
6. Stagnation of bile flow (cholestasis), associated with dilated bile capillaries, many with plugs of bile, and pigmentation of the cytoplasm of liver cells. Prolonged obstruction leads to bile duct proliferation, fibrosis, and degeneration of liver cells. The stagnation may be associated with inflammation of the tissues surrounding the duct. In addition to mechanical blockage, bacterial infection or intoxication by chemicals as different as arsenicals, methyl testosterone (Almaden and Ross, 1954), or chlorpromazine (Loftus *et al.*, 1955) may produce this response.
7. Cirrhosis, according to Popper (1954) "a chronic hepatitis with abnormalities of both parenchyma and mesenchyma, including scarring." An altered reconstruction of lobular parenchyma, due to injury of various etiology, results from two processes common to all types of cirrhosis. One is the formation of nodules in which normal arrangement of blood vessels and blood flow is altered. As a result, liver plates are rearranged. The nodules compress hepatic vein tributaries and cause elevation of blood pressure in the portal system. The second is the development of abnormal shunts between portal vein branches and hepatic vein tributaries. [For a more detailed discussion of the pathogenesis of cirrhosis see Popper's (1954) review.]
8. Inflammation, a common response to systemic disease, viral hepatitis, cirrhosis, and disease of the biliary or gastrointestinal tracts. Massive accumulations of inflammatory cells and marked proliferation of Kupffer's cells may be responsible for some of the chemical changes in blood occurring in liver disease.

Infectious disease of the liver, such as viral hepatitis, is characterized by degeneration and necrosis and regeneration of parenchymal cells as shown by variations in size and staining characteristics. Necrosis and complete disappearance of cells and destruction of the normal architecture of the lobule by scar tissue may follow. Scavenger cells, histiocytes, lymphocytes and plasma cells infiltrate the damaged regions. Edema may occur and, together with the disorganization of structure, contribute to decrease the blood supply. Regeneration of the parenchymal cells can occur rapidly and produce an astonishingly large mass of cells within as little time as 24 to 48 hours. Regeneration is favored by a well-maintained

blood supply, and lacking this, fails to occur (Grindlay and Bollman, 1952).

Viral hepatitis ordinarily runs its course within 1–3 months with apparently complete recovery. It may however, become chronic in about 1 per cent, persisting for many years, at times in an asymptomatic form. Formerly, viral hepatitis was often mistaken for a disease of the bile ducts and the term "catarrhal jaundice" in the older literature is a result of this confusion.

The term cholangiolitic hepatitis has been applied by Watson and Hoffbauer (1946) to a syndrome in which inflammation of the smaller bile passages predominates. It differs strikingly in its effect on the chemical composition of the blood from those diseases that involve primarily the parenchymal cells. Chemical changes are similar to those found in biliary obstruction and all the resources of medical knowledge may be required to make a differentiation. Abscesses and cysts of the liver caused by infections or parasites may lead to significant changes in liver function (Brem, 1955).

Deposition of fat in the liver may occur as part of a general adiposity resulting from overnutrition, as a result of dietary deficiencies, or as an effect of the action of toxic substances. Dible (1951) has recently evaluated the significance of liver fat and has demonstrated experimentally that liver fat tends to be increased in proportion to body fat. Fatty liver resulting from overnutrition has little importance insofar as the function of the liver is concerned. However, deposition of fat caused by toxic substances is evidence of a serious disturbance of hepatocellular function. Among the nutritional deficiencies associated with fatty liver are those induced by lack of betaine, choline, methionine, threonine, B_{12}, or folic acid. Low protein intakes contribute to the production of fatty livers.

Extensive deposition of fat often precedes fibrosis. Popper (1954) describes the gradual appearance of connective-tissue septa in large, fatty livers, occurring around the lobules and also extending into the lobules.

Although liver disease of nutritional origin is uncommon in the United States (if the chronic alcoholic is excepted), it is a major problem in many parts of the world, notably the tropical regions of Africa and Asia and in the Caribbean area. It may be accompanied by infiltration of fat, necrotic changes, and cellular infiltration, and in certain instances by accumulation of exudate in the liver. In certain parts of Africa, primary carcinoma of the liver may be a major manifestation of nutritional deficiency; in others, cirrhosis has been reported in four of five necropsies.

Liver damage may occur as a result of severe strains on metabolism

associated with many diseases. In infectious mononucleosis it may be sufficiently severe to constitute a grave hazard to the patient. Marked jaundice and severe alterations in liver function are seen in such patients. Malaria, lobar pneumonia, typhoid fever, various anemias, syphilis, and cholera serve as other examples. Diabetes and thyrotoxicosis also may lead to marked liver damage. Surgical operation and anesthesia cause impairment of liver function in some patients (Fairlie and others, 1951). Liver failure may occur in pregnancy.

A failing heart and the resulting accumulation of blood in the liver and other viscera (chronic passive congestion) may lead to marked impairment of the efficiency with which the liver functions.

Proliferation of the connective tissue of the liver may result from liver disease of infectious, nutritional, toxic, hypoxic, or neoplastic etiology, or it may occur spontaneously because of diminished blood supply resulting from circulatory and other factors. The overgrowth of connective tissue in turn leads to disorganization of the liver structure and this again to further interference with the blood supply. The end result is a shrunken liver consisting largely of connective tissue, and with a markedly decreased mass of parenchymal, reticuloendothelial, and vascular tissue. The designations *portal cirrhosis*, *atrophic cirrhosis*, and *Laennec's cirrhosis* have been used at various times to denote such scarred livers. Although not all scarred livers fit the pathologist's definition of Laennec's cirrhosis, this descriptive term appears to be preferred at present. Laennec's cirrhosis accounts for a large proportion of the liver disease encountered in American hospitals.

Cirrhosis of a completely different etiology, differing also in its morphologic and chemical characteristics, appears after biliary obstruction has persisted for long periods. Known as biliary cirrhosis, it is relatively uncommon. This condition may occur spontaneously also, its etiology being unclear.

Obstruction of the bile ducts often causes jaundice, which may be attributed erroneously to liver disease. It is essential to distinguish jaundice due to biliary obstruction from that caused by liver disease or excessive destruction of blood, because obstruction requires surgical treatment, whereas surgery may not be well tolerated by the patient with liver disease or be needed by the patient who is hemolyzing his erythrocytes. Gall stones entering the common bile duct are the usual cause of biliary obstruction. Other causes include neoplastic disease of the ducts, especially of the ampulla of Vater, or carcinoma of the head of the pancreas. Stricture of the ducts may follow infection, surgical exploration, or other

trauma. Disease of the gall bladder frequently is complicated by liver damage. The pancreas often shows evidence of being involved. Failure to establish the presence of biliary obstruction and to correct it may lead eventually to biliary cirrhosis.

Carcinoma or other types of neoplastic disease of the liver may or may not be accompanied by jaundice. Often such lesions present few signs to the physician. Obstruction of the bile ducts within the liver by neoplasm presumably brings about the rise in alkaline phosphatase activity in serum, which provides one of the few diagnostic aids available.

Damage to the liver may be caused by a large number of chemicals and drugs. Carbon tetrachloride has been extensively used for production of experimental liver damage and probably has been involved more often than realized as an insidious cause of clinical liver disease. Atophan, various sulfonamides, para-aminobenzoate, testosterone, arsenicals, and others have been implicated. The effect on liver function, as measured by laboratory studies, may resemble that observed in biliary obstruction rather than parenchymal liver disease.

Liver disease may affect the metabolism and functions of other organs. Effects upon the brain are especially noteworthy. Continuing liver disease of maximal severity may eventually lead to loss of consciousness or convulsions, and the electroencephalogram may show the characteristic pattern seen in the syndrome known as hepatic coma. Hepatic coma is frequently but not necessarily fatal. Recovery may occur spontaneously but also has been attributed to a variety of therapeutic agents. Among treatments reported as successful are injection of nicotinic acid, thiamine phosphate, vitamin B_6, multiple B vitamins, sodium glutamate, and glucose. Exchange transfusion and oxygen therapy also have been used.

Impairment of kidney function commonly accompanies liver disease, and may become a grave problem. The coexistence of hepatic and renal failure is often referred to as the "hepato-renal syndrome." Whether this is a clinical entity has been questioned by many. Lichtman (1954) points out that renal complications in liver disease vary in their pathogenesis. Patek, Segal, and Bevans (1951) find that intercapillary glomerulonephritis occurs much more frequently in cirrhotic patients than in the ordinary population. Farquhar (1949) has described the deterioration of kidney function occurring in viral hepatitis.

CHEMICAL PHYSIOLOGY OF LIVER DISEASE

The list of chemical disturbances observed in liver disease is a long one and will become lengthened as metabolism in liver disease is studied more

intensively. Knisely (1951) has listed 18 major asserted chemical functions of the liver with 75 subheadings. Many of these cannot be discussed here for lack of space.

Carbohydrate Metabolism

Studies of hepatectomized animals by Mann (1927) and his collaborators showed conclusively the vital importance of the liver for maintenance of the blood glucose concentration. Although hypoglycemia severe enough to cause symptoms is not a common complication in acute parenchymal liver disease, it does occur in cirrhosis of the Laennec type with sufficient frequency to require that measurement of fasting blood glucose concentrations be included in the study of such patients. Blood sugar concentrations as low as 25 mg./100 ml. are not unusual. A diagnosis of islet cell adenoma of the pancreas occasionally is erroneously made in cirrhotic patients because of recurrent hypoglycemia (Conn *et al.*, 1938). Mellinkoff and Tumulty (1953) describe the occurrence of hypoglycemia in patients with liver disease, including some of those with relatively moderate liver involvement.

Waife *et al.* (1951) showed that insulin resistance occurs in cirrhosis, the fall in blood sugar after a standard insulin injection being delayed as compared with that of healthy individuals. However, hypoglycemia following the insulin persisted for a much longer time than in healthy controls. Thus a markedly impaired ability to mobilize glucose in response to hypoglycemia may be deduced. Injection of epinephrine causes a smaller rise in blood sugar of patients with liver disease than it does in healthy subjects (Geill, 1943; Kinsell and associates, 1949). Hillman (1949) reports this test to have doubtful value in the study of liver function.

Glucose administered to patients with liver disease often causes a greater and more persistent rise in blood glucose than it does in healthy individuals. (Conn *et al.*, 1938; Campbell and Tagnon, 1946). Smith, Ettinger, and Seligson (1953) have studied the metabolism of fructose and of glucose in patients with liver disease. They found evidence of impaired ability to utilize both, but not of sufficient consistency to enable application as diagnostic or functional tests.

Decreased utilization of galactose in liver disease has provided the basis for one of the earlier tests of liver function (Bauer, 1906). The measurement of galactose excretion in urine originally used has been replaced by measurement of blood galactose concentrations (Althausen, Lockhart and Soley, 1940; Althausen, 1949; Zieve, Hill and Nesbitt, 1950). Colcher,

Patek and Kendall (1946) have described an intravenous galactose tolerance test by which the quantity of galactose removed per minute was found to be markedly decreased in liver disease. The cause of the elevated blood galactose and increased output in urine characteristic of severe liver disease has not been established, but presumably it represents interference with the conversion of galactose to glucose. An idiopathic defect in galactose metabolism involving a galactose kinase has been studied by Greenman (1950). The liver is thought to be the source of the enzyme.

Methods for Study of Disturbances of Carbohydrate Metabolism in Liver Disease

Blood sugar determinations after an overnight fast generally will enable detection of hypoglycemia. A diet very low in carbohydrate accentuates the tendency toward hypoglycemia. (Conn. *et al.*, 1938). Glucose tolerance measured by standard 3- or five-hour technics may be used but, as indicated above, offers little useful diagnostic information. Measurement of phosphate concentrations in serum also fails to differentiate clearly between the several causes of impaired glucose tolerance. The galactose tolerance procedures are discussed above. Further studies by means of these improved technics are needed for appraisal of this procedure. The recent literature gives little reason to believe that fructose tolerance tests would be of value in diagnosis of liver disease.

Serum Cholesterol and Lipids in Liver Disease

The serum lipids often show marked changes in diseases of the liver and biliary tract. Although serum cholesterol concentration and the partition between free and esterified cholesterol is most frequently studied, neutral fat and especially phospholipid may also show marked changes. Relationships between free and esterified cholesterol and between free cholesterol and phospholipid, which in health are maintained within narrow limits, are subject to striking disturbances in severe liver disease. Liver disease in which injury to the parenchymal cells is severe is characterized by lowered lipid concentrations which often fall below the minimal concentrations observed in normal individuals of the same age. The onset of jaundice in viral hepatitis is soon followed by falling serum cholesterol and phospholipid concentrations. The decrease of the esterified cholesterol is striking. Both concentration of esterified cholesterol and the percentage of the total cholesterol esterified are lowered. If liver damage is very severe, esterified cholesterol may become undetectable. Recovery is accompanied by rising concentrations of the esteri-

fied cholesterol in serum. Free cholesterol often is increased at the same time so that moderately elevated concentrations may exist during the stage of remission.

Lecithin, but not cephalin, concentrations are also increased in serum at this stage (Peterson, 1953). Alterations in serum lipid concentrations and the interrelationships of the various lipids have been described by Man *et al.* (1945), Hoagland and Shank (1946), Havens (1948), and Billing *et al.* (1955). Studies of the lipoproteins of sera of patients suffering from liver disease show that these also are involved in the general disturbance of lipid metabolism. Pierce and Gofman (1951) observed a marked increase in the lipoproteins of the class S_f 10–20. Pierce *et al.* (1954) described high values in the S_f 0–12, 20–100, and 100–400 classes also. The serum lipoproteins of hepatectomized dogs remained unchanged for 6 hours, and then decreased despite increasing hemoconcentration (Lewis, Page, and Thomas, 1953). Nikkila (1953) demonstrated a decrease in α_1 and an increase in β-lipoprotein by the use of zone electrophoresis in hepatitis and also in biliary obstruction. Cirrhosis of the Laennec type, is characterized by low serum lipid concentrations, especially when atrophy of the liver is extensive. Serum cholesterol concentrations of less than 100 mg./100 ml. are quite common. The proportion of esterified cholesterol also is lowered, although exceptions exist. Low phospholipid concentrations also are the rule. Cayer and Cornatzer (1950) found the rate of phospholipid synthesis in cirrhosis to be decreased and this may account for the low values in serum.

Billing *et al.* (1955) found a correlation between total liver lipids and total serum lipids, and also between total liver lipids and serum total fatty acid, phospholipid, and total and free cholesterol in 22 patients with cirrhosis. No correlation could be established by the study of a group of patients with viral hepatitis or in fatty vacuolization, focal necrosis, or granuloma. Colwell (1954) observed that an excessive deposition of fat occurred in the liver during convalescence from viral hepatitis if the patient gained much weight during the convalescence period. Such patients ate diets rich in carbohydrate and fat and relatively poor in protein.

Biliary obstruction regardless of cause is characterized by elevated concentrations of serum lipids. Extremely high concentrations, among the highest known to occur due to any cause, are encountered in patients with biliary obstruction of long duration or with biliary cirrhosis. Ahrens *et al.* (1950) in a review dealing with biliary cirrhosis describe a disproportionate increase in serum phospholipid concentration. They have also

clarified the relationship of elevated serum lipid concentrations to xanthelasma and xanthomatosis.

Cholangiolitic hepatitis and toxic hepatitis of the type caused by chemicals and drugs also are associated with elevated serum lipid concentrations.

Some use is made of serum cholesterol analyses for differentiation of primarily parenchymal from primarily biliary lesions in jaundiced patients. However, such analyses offer little information that cannot be obtained more easily and dependably by other methods. Not only do some patients with biliary obstruction fail to show a rise in concentration of serum cholesterol exceeding the broad range of concentrations found in healthy individuals, but abnormally elevated serum cholesterol concentrations occur in many patients with infectious hepatitis or other predominantly parenchymal types of liver disease. Such increased levels are observed especially as regeneration of the liver proceeds.

The finding of low concentrations and ratios of esterified to total cholesterol in a jaundiced patient is strong but not conclusive evidence for parenchymal liver involvement of severe degree. Serial studies of the esterified cholesterol concentration provide means for estimating changes in the state of the liver in parenchymal liver disease of maximal severity. They do not however, reliably indicate the prognosis. Esterified cholesterol measurements are useful in the study of such patients because more sensitive tests, such as bromsulfalein retention, become maximally positive when there is still a substantial amount of functioning parenchymal tissue remaining. On the other hand, for study of patients with less severe liver disease, less elaborate and time-consuming methods than esterified cholesterol determinations are more satisfactory. As an indication for determination of esterified cholesterol, the presence of jaundice or of a marked elevation of serum bilirubin serves reasonably well. Measurement of esterified cholesterol concentration is useful for the preoperative study of patients with biliary tract disease.

Zieve (1953) has demonstrated that the lowered esterified cholesterol concentrations in patients with liver disease and obstruction of the common bile duct are correlated with the degree of jaundice. Although the proportion of cholesterol esterified shown in his data varied widely for any given bilirubin concentration, a fact that suggests some degree of independence, Zieve's study has helped to prove what many workers have long suspected—that esterified cholesterol measurements make a rather limited contribution to the study of patients with liver disease.

The cause of the lowered concentrations of serum lipid observed in

many patients with severe liver disease is not known. Contributing factors include (1) the lowered intake of lipid brought about by prescription of diets high in carbohydrate and low in fat for patients with liver disease; (2) decreased synthesis of fat and of other lipids because of reduced mass of parenchymal cells, impaired blood supply and other related mechanical factors leading to impaired efficiency of biochemical transformations; (3) a diminished supply of supplementary factors such as labile methyl, choline, etc., because of diminished intake and decreased synthesis, these together with other factors, leading to (4) deposition of fat in the liver; and (5) impaired absorption of fat from the gastrointestinal tract. Steatorrhea is a frequent finding in patients with liver disease. Gross *et al.* (1950 b) attribute this to deficiency of bile salts, for they were unable to demonstrate a lack of digestive enzyme secretion by the pancreas in patients with viral hepatitis.

The decreased esterification of cholesterol occurring in severe liver disease, when there is extensive destruction or replacement of the parenchyma, also is unexplained. It may be a manifestation of a general decrease in esterase activity since cholinesterase of serum is also depressed. Bollman (1950) has pointed out that the intestinal mucosa is an active site for esterification of cholesterol and that it adds by way of the lymph each day twice the amount of cholesterol ester found in the plasma at any time. However, Friedman and Byers (1955) offer strong evidence that the liver is the organ that chiefly synthesizes and removes cholesterol esters.

The cause of the elevated concentration of lipid in serum, so typical of biliary obstruction, is also obscure. The bile does not appear to be an important route of lipid excretion. Much of the cholesterol and probably all of the phospholipid excreted in the bile is reabsorbed. Balfour (1947) found that the rate of production of phospholipid was increased in biliary obstruction and that the rise in concentration was proportional to the increase in rate of its production. Rosenman, Friedman, and Byers (1952) have postulated that cholesterol concentration in serum rises as a response to an increase in concentration of bile acid (cholate). They believe that changes in concentration of the latter are accompanied by changes in serum cholesterol which serves perhaps to counteract the toxicity of cholate for tissues. Byers, Friedman, and Michaelis (1951) find that the liver itself is the source of the extra cholesterol. Frederickson *et al.* (1954) also observed a nearly 20-fold increase in cholesterol synthesis in rats following ligation of the bile duct. The close relationship maintained between free cholesterol and phospholipid might then be expected to lead to increased concentrations of the latter.

Information concerning bile acids in liver disease is scanty and unsatisfactory. It is to be hoped that recent improvements in the methods for bile acid determination in serum and bile will overcome this deficiency. Studies of fistula bile following release of biliary obstruction in patients showed cholate to be absent from the bile for a number of days (Ravdin *et al.* 1933). The crude methods available in the past for estimating bile acid concentrations in serum indicate that bile acid enters the blood stream to attain concentrations of 10 to 20 mg. per 100 ml. in the presence of biliary obstruction. Elevated values may occur also in liver disease affecting the parenchyma predominantly (Sherlock and Walshe, 1948).

Methods for Study of Disturbances of Lipid Metabolism in Liver Disease

Serum cholesterol and cholesterol ester concentrations may be determined by the methods of Abell *et al.* (1952) or of Sperry and Webb (1950). For phospholipid concentrations the method of Zilversmit and Davis (1950) is convenient. A rapid and simple method for detecting total lipid of serum, the phenol turbidity, has been described by Kunkel, Ahrens, and Eisenmenger (1948). De la Huerga *et al.* (1953) have described a rapid turbidimetric method for total lipid in serum that depends upon liberation of lipid in fine suspension by a reagent consisting of dioxane and dilute sulfuric acid. If a more accurate method is required, that of Sperry and Brand (1955) may be used.

Nitrogen Metabolism

Several studies of the nitrogen metabolism of patients with liver disease have failed to demonstrate any marked imbalance. Kinsell *et al.* (1948) found the nitrogen balance of 2 patients with chronic hepatitis to be positive. Gross *et al.* (1950 a) found a slightly positive nitrogen balance in 7 patients diagnosed as having acute hepatitis and in one with chronic hepatitis. They cite the work of Fawitzsky in 1889 who studied 6 patients with Laennec's cirrhosis and came to a similar conclusion.

The liver is the principal site for transformation of amino acids by synthesis, transamination, etc., into those forms specifically needed. It carries out such transformations less effectively when diseased or otherwise injured.

Amino acid concentrations of plasma (Flock *et al.*, 1953) and of brain and muscle (Flock, Block, *et al.*, 1952) are increased substantially after complete removal of the liver. In liver disease the concentration of amino acid in plasma may be increased in patients with severe involve-

ment of the liver. Hsia and Gellis (1954) found that of 19 children ill with viral hepatitis, about half had moderately elevated concentrations of amino acid in plasma. About two thirds excreted increased amounts in urine. Walshe (1953) found by means of chromatographic methods that some adults showed little change in plasma amino acid in hepatitis, whereas marked changes occurred in others. However, Murphy *et al.* in 1949 failed to demonstrate an increase in plasma amino acids in a group of patients with liver failure. Mellinkoff *et al.* (1954) also found only moderately increased concentrations in viral hepatitis, although the results are somewhat questionable because of the use of serum for analysis instead of plasma. After a meal containing 20 Gm. of protein, amino acid concentrations decreased in serum of patients with hepatitis. In healthy persons the opposite occurred. The cause of the unexpected behavior of the amino acids in hepatitis is obscure, but the suggestion is offered that it may be the effect of insulin present in increased concentration in the tissues because of a diminished rate of destruction of insulin by the damaged liver. Insulin was shown to cause an abrupt fall in amino acids of plasma after hepatectomy (Flock *et al.*, 1953).

Several reports indicate that methionine concentration is increased considerably in plasma in viral hepatitis (Kirsner *et al.*, 1950; Schreier, 1951; Balch *et al.*, 1954; Kinsell *et al.*, 1948). Kinsell *et al.* (1948, 1949) also demonstrated a delayed rate of removal of injected methionine from the blood stream. Walshe (1953) and Walshe and Senior (1955) describe the occurrence of high cystine concentrations in plasma in cirrhosis, with highest values occurring in hepatic coma.

Mitchell, Butt, and Code (1954) found elevated blood histamine concentrations in patients with liver disease. They noted a correlation between pruritus and the extent of the elevation.

The excretion of certain amino acids in urine is increased substantially in parenchymal liver disease (Dunn *et al.*, 1950; Gabuzda *et al.*, 1952; Walshe, 1953). Among those affected in this way are cystine, methionine, taurine, β-aminoisobutyric acid, methyl histidine, and tryptophan. The excretion of some amino acids is lowered. Among these are lysine, histidine, and isoleucine. Dent and Walshe (1951) describe several different patterns of aminoaciduria occurring in liver disease.

Walshe (1953) identified more than 30 amino acids in urine following massive necrosis of the liver. He found the output of amino acids in urine to undergo greater change than did the concentration in plasma. Relatively moderate gains in concentration in plasma led to a marked increase in the output of amino acids in urine. Thus, the change in plasma con-

centrations of amino acids with high renal clearances often was slight compared with the change in the amounts found in urine. Amino acids with lower renal clearances, including methionine, phenylalanine, and tyrosine, show much more marked changes in plasma concentrations.

Dent and Walshe (1954) state that patients with biliary obstruction, secondary carcinoma of the liver, and liver infiltrations had no abnormality of amino acid metabolism. The extent of abnormality in parenchymatous disease corresponded roughly to the extent of the disease.

Walshe (1951) detected methionine sulfoxide in chromatograms of the plasma of a patient dying of hepatic coma. This compound is a glutamic acid antimetabolite and the suggestion was made that it might be inhibiting the utilization of glutamic acid by the brain. Interference with the metabolism of a substance having such a vital role in brain metabolism would have important consequences. However, it may be an artefact.

Ethanolamine was found in excessive amounts in urine of patients with liver disease by Dent and Walshe (1951, 1953). One patient with a huge hepatoma excreted 0.5 to 1.0 Gm. per day. The presence of large amounts of ethanolamine, a precursor of lecithin, may be related to the failure of phospholipid synthesis referred to above.

In Wilson's disease (hepatolenticular degeneration) amino acid output in urine is substantially increased, whereas little or no change can be detected in concentrations of blood amino acids (Dent and Walshe, 1951).

Some of the tests employed for study of liver function have as their basis the impaired metabolism of amino acid. Among such is the tyrosine tolerance test of Bernhart and Schneider (1943). One of the factors causing delayed excretion of hippuric acid in Quick's test (1933) is said to be the decreased ability to mobilize glycine for conjugations.

The elevated amino acid concentration in blood contributes to the elevation of nonprotein nitrogen characteristic of patients with severe liver involvement. Concentrations of other substances, including creatine, creatinine, uric acid, ammonia, as well as the undetermined nitrogen, also may be elevated. Urea concentrations may be increased, within normal limits, or decreased, the latter being a rare occurrence. The elevation of nonprotein nitrogen is mainly a result of the impairment of kidney function. However, urea nitrogen may not be elevated in proportion to the creatinine and NPN concentrations. In part this is due to impaired efficiency of the reactions involved in its synthesis. A second factor is the lowered protein intake often prescribed for patients with liver disease. Conclusions concerning the kidney function of patients with severe disease of the liver are, therefore, likely to be more reliable if

based on creatinine or nonprotein nitrogen determinations than if based on blood urea nitrogen.

Creatine concentrations are markedly increased in the serum of some patients in hepatic coma (Reinhold, unpublished observations). The impaired metabolism of muscle in such patients, with resulting losses of creatine, is thought to be the main cause. Renal failure alone is not responsible. Failure of methylation may contribute.

Blood Ammonia in Liver Disease

The liver is the site of numerous reactions concerned with ammonia metabolism. These include deaminization of amino acids and adenylic acid and synthesis of glutamine. Blood ammonia concentrations in hepatectomized dogs were found to rise rapidly (Gordon, Freeman, and Farmer, 1952), confirming earlier work by Bollman and Mann in 1930. Interest in blood ammonia in liver disease has been revived recently by the finding that patients suffering from cirrhosis, who had received ammonia-containing ion exchange resins, at times became stuporous with signs suggesting those of hepatic coma (Gabuzda, Phillips, and Davidson, 1952). Blood ammonia concentrations were substantially increased (Schwartz *et al.*, 1953). Fuld (1933), Kirk (1936) and others previously have shown that the ammonia content of blood is increased in patients with severe liver disease. Seligson (1953) reported that not all patients in hepatic coma had elevated blood ammonia concentrations.

Riddell and McDermott (1954) found higher blood ammonia concentrations in blood of patients with cirrhosis than in that of normal persons, especially in the period preceding coma. However, a decrease in blood ammonia concentrations of such patients was not necessarily accompanied by an improved clinical condition. Administration of ammonium compounds to cirrhotic patients caused a greater rise in blood ammonia concentrations than in healthy persons (Traeger, *et al.*, 1954). Ammonia concentrations in cerebrospinal fluid exceeding 105 μg./100 ml. were associated with mental disturbances (McDermott, Adams, and Riddell, 1955).

The neurologic effects of elevated ammonia concentrations in the body fluids are of unusual interest. They have been described by Adams and Foley (1953) and by McDermott and Adams (1954).

Bessman and Bessman (1955) found that ammonia is converted in brain and muscle to a bound form. Arterial blood contained somewhat higher concentrations of ammonia than did venous blood returning from the brain. They believe that the uptake of ammonia by the brain causes a

diversion of α-ketoglutarate to glutamate. A deficiency in the production of the former is thought to interrupt the citrate cycle and may impair cerebral metabolism. A study by Nelson and Seligson (1953) showed that ammonia enters the circulation mainly from the portal vein, kidneys, and muscles. In shock the liver fails to remove it completely and the blood ammonia level rises.

Studies of the ammonia metabolism of experimental animals (Mann *et al.*, 1954) and of patients suffering from liver disease, especially cirrhosis (Schwartz *et al.*, 1954), have led to a critical re-evaluation of the role of high protein intake in raising blood ammonia concentrations. High protein intake tends to raise blood ammonia concentrations and low protein intake to lower them. It appears from this that the protein intake should be adjusted to the capacity of the patient to dispose of ammonia.

These observations, together with those of Nelson and Seligson (1953), strongly implicate the intestine as the major source of the blood ammonia. Parnas and Klisiecki (1926) came to a similar conclusion nearly 30 years ago. In the normal person most of the ammonia is removed by the liver. However, the considerable proportion of the blood that flows around instead of through the liver of the cirrhotic delivers the ammonia into the general circulation and so raises the ammonia concentration. White *et al.* (1955) believe that there is a defect in hepatic metabolism of ammonia in addition to the enteral factor.

Blood ammonia concentrations may be measured either by the method of Conway (1935) [*see also* Conway and Cooke (1939) and McDermott and Adams (1954)] or by the method of Seligson [described by Reinhold and Gentzkow (1955)]. Although the two methods differ in results, either procedure is capable of providing useful information about blood ammonia concentrations. For both theoretical and practical reasons that of Seligson is the method of preference.

Plasma Proteins in Liver Disease

The liver has a dominant role in plasma protein synthesis. It is the known source of plasma albumin and fibrinogen (Miller, Bly, Watson, and Bale, 1951). Probably proteins other than fibrinogen involved in the clotting of blood also originate in the liver. It is the source of important components included in the alpha and beta globulins (Roberts, 1953; Roberts and Brunish, 1953). The liver also is involved in synthesis of gamma globulin, although there is much evidence indicating that synthesis of gamma globulin is largely extrahepatic.

Albumin concentrations in serum are lowered in patients suffering from cirrhosis, in viral hepatitis during its clinically active stages, in

nutritional liver disease, and in neoplastic disease involving the liver. Many authorities consider it to be among the most dependable measurements available for establishing the presence of liver disease (numerous other causes of low albumin concentration usually can be excluded without difficulty) and for following its clinical course. For this purpose it is superior to total serum protein concentration because changes in albumin are commonly masked by an equal and simultaneous rise in globulin so that total protein remains unchanged. The changes occurring in serum proteins of a volunteer in whom viral hepatitis was induced are shown in Fig. 3.

The causes of the decreased albumin concentration include (1) impaired synthesis because of decreased mass of parenchymal tissue; (2) losses by transudation into ascitic and edema fluid; (3) losses by hemorrhage, a frequent complication of cirrhosis; (4) increase in plasma volume (in cirrhosis) and (5) low protein intake.

The lowered concentration of serum albumin is one of the factors re-

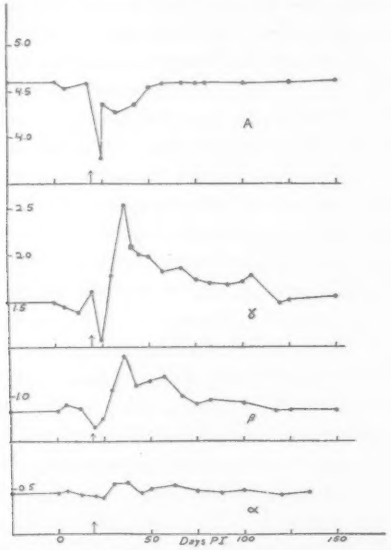

Fig. 3. Changes in serum proteins occurring in a human volunteer during the course of viral hepatitis. At 0 days, this volunteer ingested material containing virus obtained from a patient suffering from infectious hepatitis. The arrow indicates the appearance of clinical symptoms. Note the fall in albumin concentration and the sharp rise in gamma and beta globulin concentrations with gradual return to the original values. Protein measurements were made by the moving boundary method at *p*H 7.6 in phosphate buffer. (From unpublished studies by Neefe, Chambers, Gilman, and Reinhold.)

sponsible for the occurrence of flocculation in the cephalin-cholesterol flocculation and related tests. A change in constitution of albumin and α-globulin probably contributes. A second major factor is a rise in concentration of γ-globulin. Yet another variable is a rise in stabilizing action associated with biliary obstruction (Ducci, 1950). The physicochemical basis for the flocculation and turbidity tests has been discussed by Saifer (1952).

An increase in γ-globulin accounts for much of the increase in total globulin of serum. Whereas in healthy individuals γ-globulin reaches a maximum of 1.6 Gm./100 ml., in liver disease concentrations double this are common, and concentrations five or more times the maximal normal occur in some patients with hepatitis. Zimmerman, Heller, and Hill (1951) observed a globulin concentration of 9.9 Gm./100 ml. in a patient with subacute hepatic necrosis. Values as high as this have been encountered on several occasions in the writer's laboratory. [*See also* Kunkel, Ahrens, *et al.* (1951).]

The marked rise in γ-globulin occurring in liver disease is similar to that occurring in many other diseases. Robertson (1950) has described extremely high γ-globulin concentrations as a manifestation of sensitization to drugs, and Carter (1949) described equally high γ-globulin concentrations as a result of trichinosis. Teilum (1948) considers the rise to be part of a general response to sensitizing agents. The rise is associated with a marked proliferation of plasmacytes in bone marrow to as much as 25 per cent or more of the total count. Plasmacytes and lymphocytes also accumulate in the liver and other affected areas. These and other observations have led Fagreus (1948), Bjorneboe and associates (1947), and Ehrich (1953) and others to attribute to the plasma cells the formation of γ-globulin. According to their hypothesis the elevation in γ-globulin occurring in response to stressful stimuli, among them liver disease, is a result of the proliferation of this γ-globulin-producing tissue. However, Popper (1951), Franklin (1951), and their coworkers have observed changes in the Kupffer and mesenchymal cells of the liver that correlate with increased γ-globulin concentrations in serum. They believe that these cells are the source of the extra γ-globulin. Yet another explanation has been offered by Miller (1953), who believes that the diminished synthesis of albumin and other plasma proteins leaves a surplus of amino acids which are converted into γ-globulin outside the liver.

The rise in γ-globulin in many patients suffering from liver disease is accompanied by elevation of β-globulin. Extra protein components not normally observed may appear when electrophoretic separation of serum

protein is made at *p*H 8.6, (Martin, 1949). That most frequently encounted is the H protein of Viollier (1950) observed in infectious hepatitis. Increased gamma$_1$ concentrations were observed by Franklin and associates (1951) frequently in liver disease and rarely in other conditions. These components have mobilities close to that of fibrinogen. Other abnormal components may appear within the gamma and beta fractions.

Fibrinogen concentrations of plasma fall after removal of the liver (Foster and Whipple, 1922). Stefanini (1949) found low fibrinogen concentrations in patients suffering from liver disease. Schulz (1953) observed occasional high fibrinogen values in viral hepatitis. These fell in the later stages. Low values were the rule in cirrhosis.

Alpha globulins, particularly α_1, show a tendency toward lower concentrations than those existing in health. Much of the mucoprotein of serum is included within the alpha-globulin fraction of the serum protein. Serum mucoprotein has been found by Greenspan *et al.* (1952; 1953; 1954) to be decreased by hepatitis but increased by metastatic invasion of the liver. Mandel *et al.* (1955) find that the results are not always consistent with the diagnosis, and have reservations about the usefulness of the mucoprotein measurement. However in the writer's laboratory, the serum mucopolysaccharide measurement appears to have provided valuable aid to physicians confronted with diagnostic problems in jaundiced patients.

Mucoprotein may be measured by a method such as that described by Rhees, Ellerbrook, and Lippincott (1954). Graff *et al.* (1951) showed that the somewhat laborious method for mucoprotein could be replaced by measurement of protein-bound polysaccharides, a relatively simple procedure when anthrone is used as the reagent. However, see Shetlar (1952).

The concentrations of some of the specific proteins included in the alpha globulin fraction also are decreased. Thus serum pseudo-cholinesterase is markedly decreased in many patients with liver disease. Gray, Probstein, and Heifetz, (1941) showed that serum amylase activities are decreased (exceptions occur if there is an associated pancreatitis or impaired renal function). Others have reported lowered lipase and esterase activities.

Methods for Evaluating Changes in Serum Protein

Practical methods for serum albumin and globulin determinations have been described in a recent article (Reinhold, 1953). Measurements of albumin and globulin concentrations are among the more useful available

for the study of liver disease. Zone electrophoresis on paper shows great promise as a method for study of serum proteins of patients with liver disease, Knedel (1951), Brante (1952), Satoskar *et al.* (1954) have described changes in serum protein in diseases of liver and biliary tract measured by zone electrophoresis.

Some patients, especially those with liver damage of moderate degree, do not show significant changes in serum albumin and total globulin concentrations. The quantitative measurement of γ-globulin concentrations may offer some advantage in this connection and the salting-out methods of Wolfson *et al.* (1948) or of Jager and Nickerson (1948) are available for this purpose. De la Huerga and Popper (1949) have described a turbidimetric method for measurement of γ-globulin which is rapid although less accurate than the preceding.

However, the most widely used methods for detecting changes of the type occurring in serum proteins in liver disease are the semi-empirical flocculation and turbidity tests. More than a dozen of these tests have been proposed. Among them the zinc turbidity (Kunkel, 1947), thymol test (Maclagan, 1944) and cephalin-cholesterol flocculation test (Hanger, 1939) are widely used in this country. Saifer's (1952) review may be consulted for information about other flocculation and turbidity tests and for an analysis of their mechanisms. [*See also* Maclagan *et al.* (1952) and Armas-Cruz *et al.* (1952).]

The zinc turbidity test depends upon the poor solubility of zinc compounds of γ-globulin in solutions of low ionic strength. If experimental conditions are carefully maintained, the absorbency of the suspension produced by adding to serum the zinc ion in a solution of barbital buffer will approximately measure the concentration of γ-globulin. The presence of markedly increased concentrations of γ-globulin are demonstrated by a marked increase in turbidity readings. However, smaller changes in γ-globulin concentration are not as dependably demonstrated. This is because the density of the suspension formed is governed in part by the concentration of albumin and, perhaps, of α_1-globulin in addition to the concentration of γ-globulin.

A significant difference between the zinc turbidity readings of healthy Negroes and whites has been demonstrated (Reinhold, Rawnsley, and Yonan, 1955). The average value of the former was 7.30 ± 1.78 units; that of the whites 4.97 ± 1.44 Shank-Hoagland units. This difference is sufficient to affect interpretation of the results of this test when it is applied to Negroes.

Elevated concentrations of γ-globulin are the rule in viral hepatitis,

cirrhosis of the Laennec type, and certain other types of liver disease. However, comparable elevations of γ-globulin concentrations occur in many other diseases and this lack of specificity must be considered in interpreting the results. The zinc turbidity is elevated in a few patients, particularly those suffering from chronic liver disease while the thymol and cephalin-cholesterol flocculation tests are negative. Thus it finds some use as a supplement to these ordinarily more sensitive tests. The zinc turbidity often is within normal limits when the thymol and cephalin-cholesterol tests are abnormal, whereas the reverse occurs infrequently.

Thymol Test

The thymol turbidity test was devised by Maclagan (1944). Its basis was the discovery that a saturated solution of thymol in barbital buffer produced marked turbidity when added to the serum of patients suffering from extensive liver disease. On standing, a flocculum appeared in some abnormal sera.

If satisfactory results are to be obtained, the thymol concentration and temperature, and especially the *p*H of the reagent, must be rigorously controlled.

Three methods of preparing thymol barbital reagent have been described. In addition to Maclagan's (1945) original method, de la Huerga and Popper (1949) and Yonan and Reinhold (1954) have described modifications. The directions for preparation of the reagent of Yonan and Reinhold may be found in the original version of the present review (Reinhold, 1953) and will be published shortly as a separate article. Objections to Maclagan's technic are (1) the difficulty of producing reagents of uniform quality and (2) the poor keeping qualities of the reagent. Carne (1953) and Katz *et al.* (1954) recommended the de la Huerga and Popper reagent in preference to that of Maclagan.

The temperature at which the tests are done is a factor of critical importance (Yonan and Reinhold, 1954).

Accurate measurement of thymol turbidities is difficult. Visual comparison as proposed by Maclagan lacks precision and accuracy. Photometric measurement is complicated by two major problems, (1) the optical geometry of the instrument used and (2) the preparation of a standard. Photometers of different design may give significantly different measurements. It appears that most of the work done on the clinical application of the thymol test (in the United States at least) has been done by means of the Evelyn or Coleman photometers. If other instruments are to be used, the relationship of the readings obtained to those

of the foregoing should be tested. In the writer's experience, the Beckman DU, and Klett-Summerson instruments may give results that differ markedly from those of the other two. Previously, Hoyer and Jorgensen (1952) have directed attention to the importance of the optical dimensions of the photometer.

Some of the difficulty occurs because the reaction with lipid yields particles differing in size from those formed by reaction with protein (Kunkel and Hoagland, 1947; Reinhold, 1955), and can be avoided if fasting specimens are obtained for examination. Difficulties in the turbidimetric measurements may also be diminished by use of an appropriate standard (Reinhold, 1955).

Confusion caused by the inadvertent introduction of a second unit (Shank and Hoagland, 1946) may be eliminated if results are reported in named units, e.g. "Maclagan" or "Shank-Hoagland." Two of the latter units equal 1 Maclagan unit.

Interpretation

Thymol turbidity readings are affected by ingestion of food containing fat, and measurements made by the use of serum collected from persons in fasting state are more easily interpreted. Reinhold and Yonan, in a study to be published, found thymol turbidities of 68 out of 71 healthy persons who were fasting to read below 4.0 Shank-Hoagland units when a reagent buffered at *p*H 7.55 was used. After a meal, the limit rose to 4.8 units. If the reagent was buffered at *p*H 7.80, readings were roughly 20 per cent lower when sera of healthy persons were tested. Differences between the readings of the reagents buffered at the two different *p*H values may become much greater when pathologic sera are studied.

Mateer *et al.* (1947) introduced the thymol-barbital reagent buffered at *p*H 7.55 with evidence that it was more sensitive than the reagent buffered at *p*H 7.80. Neefe *et al.* (1950) and Latner and Pendleton (1949) have confirmed this finding. Maclagan (1945) appears to have used a reagent at a *p*H of about 7.65 instead of 7.80 as he stated. Since the limits of normal as well as the behavior of the reagent in pathologic sera is directly dependent upon *p*H, it is important that the physician be informed concerning the reagent used. This applies also to the units used for reporting results.

Infants have lower thymol turbidities than adults (Desmond *et al.*, 1949). According to Gellis and Hsia (1953) flocculation tests are not reliable for study of infants aged less than 6 months.

The thymol turbidity test ranks high among liver function tests in its ability to reveal presence of liver disease. It is among the tests frequently positive in viral hepatitis, and is particularly useful during the recovery period for evaluation of progress. Although patients with cirrhosis may show elevated thymol turbidity, often it may fail to become positive in cirrhosis either of the Laennec or biliary type. An evaluation of a group of tests for the purpose of finding which were most effective for detecting the subclinical form of hepatitis in carriers of the virus led to the selection of the thymol test (Fitch *et al.*, 1955; Reinhold, 1955; Kassouny *et al.*, 1955).

The thymol turbidity is generally within the limits of normal in patients suffering from biliary obstruction, if this is of relatively recent origin. Occurrence of elevated thymol turbidity in a patient diagnosed as having biliary obstruction should cause the clinician to re-evaluate carefully the evidence for the diagnosis. On the other hand, lesions of the biliary tract after several months may produce sufficient damage to the liver to cause the thymol turbidity to become positive. Even in these circumstances, however, it is unusual for it to be abnormal. Ducci (1950, 1951) has studied the "stabilizing" action of serum of patients with biliary obstruction on the turbidity tests. The high concentrations of the bile salt-phospholipid-cholesterol complex and perhaps of mucoprotein in serum, characteristic of biliary obstruction, would by dispersing action inhibit the formation of the aggregates causing turbidity and flocculation.

The changes in serum responsible for elevated thymol turbidity readings are complex and difficult to study because the reagent reacts not only with certain proteins but also with certain lipids and lipoproteins. Recant *et al.* (1954) and Cohen and Thompson (1947) reported that β-globulins were the principal reactants in serum. Others have found that γ-globulins are predominant (Maclagan and Bunn, 1947; Ralli *et al.*, 1949; Marrack *et al.*, 1950; Havens and Williams, 1949; Reinhold, 1955). Kibrick *et al.* (1952) actually use γ-globulin added to serum as a standard. A third group believes that both β- and γ-globulins react (Kunkel and Hoagland, 1947; Martin, 1948, 1949; Albertson *et al.*, 1950).

Thymol turbidity may be increased in any disease characterized by marked elevation of γ-globulin. This may occur quite independently of appreciable liver involvement (Carter and Maclagan, 1946). Among the diseases which may be accompanied by elevated γ-globulin are lupus erythematosus, multiple myeloma, lymphogranuloma, sarcoidosis, tuberculosis, diseases due to parasites, and sensitization to drugs. Although liver damage may occur in these and other diseases, either intrinsically

or as a complication, it is necessary to confirm its presence by recourse to other liver function tests not directly dependent upon changes in serum protein.

Increased concentrations of lipid in serum may cause significant elevation of thymol turbidity (Shay *et al.*, 1947; Linder *et al.*, 1948; Popper *et al.*, 1948, 1949; Babb and Pedrazzini, 1949; Katz *et al.*, 1954; Reinhold, 1955). The effect of different lipids varies. Neutral fat in the large aggregates present in serum after a fat-containing meal are especially effective in raising the thymol turbidity reading. The effectiveness of the fat diminishes as the serum clears. Other lipids react in different ways (Shay *et al.*, 1947). Dekema (1951) states that phospholipids are among the serum constituents that react with the thymol-barbital reagent to form turbidity. However, experiments carried out in the writer's laboratory suggest that phospholipid at times may have the effect of decreasing turbidity. According to Maclagan (1948) addition of cephalin had a variable effect on thymol turbidity but decreased flocculation.

The significance of elevated thymol turbidity readings of sera rich in fat (lactescent) undoubtedly differs from that of elevated readings in sera of fasting persons. The relationship of such readings to liver disease is not established; however, liver disease, and especially viral hepatitis at certain stages, may be associated with disturbed tolerance for fat (*see* Reinhold, 1955, for references).

Estimation of serum lipid concentrations by means of the phenol turbidity method of Kunkel or of de la Huerga *et al.* (1953) has been used to evaluate the degree of lipidemia and thereby to provide some estimate of the extent of the effect of lipid.

Thymol Flocculation

Flocculation occurs more frequently when the thymol turbidity readings are elevated but it may occur when there is little or no rise in turbidity. Clinical experience also suggests that the occurrence of flocculation depends upon some additional or different factors (Neefe, 1946). In general, the significance of a positive flocculation test is the same as for abnormal turbidity. The flocculation test is less frequently positive than is the turbidity test; however, false positive tests are uncommon and the occurrence of thymol flocculation thus may be accepted with a higher degree of confidence as evidence of liver damage.

The serum of healthy individuals shows no flocculation. Flocculation graded 1 plus or more, therefore, is abnormal.

Cephalin-Cholesterol Flocculation (Hanger, 1938)

Although rough estimates of flocculation usually suffice, several quantitative methods have been described for measuring either the degree of clearing (Kibrick *et al.*, 1952) or the amount of cholesterol in the precipitate (Saifer, 1948; Jennings *et al.*, 1953). However, the principal difficulty continues to be the variation in sensitivity of the reagent as purchased. Although the manufacturers are at present supplying preparations that are greatly improved, the writer encountered one excessively sensitive lot during the past year. Thus the need for standardization of each lot continues. Standardization consists of testing 10 to 15 sera from healthy persons and an equal number from patients suffering from liver disease. A suitable reagent will not form a flocculum in the normal sera but will show flocculation in a high proportion of the sera of patients suffering from liver disease. Once a reagent has been standardized, subsequent lots can be tested by comparison with the acceptable reagent before the latter is used up. It is helpful also to include a sample of a standardized pooled serum with each day's run. Frozen serum may be used for this purpose, care being taken to thaw it rapidly at 45° to avoid fluctuations in turbidity caused by lack of uniform technic.

The photosensitivity of the test as carried out with reagents available 10 years ago was striking (Neefe and Reinhold, 1944; Moses, 1945). Its cause never was established. Torres (unpublished observations, 1952) working in the writer's laboratory was no longer able to demonstrate this sensitizing effect of light on cephalin-cholesterol flocculation of serum with reagents currently on the market. However, Bassir and Hall (1955) report its occurrence in sera of patients with liver disease and also those with no clinical evidence of liver disease. Difco antigen was used by both laboratories, and it appears unlikely that the reagent is responsible for the discrepancy. The technic of Torres differed from that of Neefe and Reinhold by inclusion of Merthiolate as a preservative. It is possible that this alters the photosensitive groups.

Interpretation

It is essential for the physician to know the sensitivity of the reagent used, since in different laboratories the readings of the sera of healthy persons and of patients without liver involvement may vary from negative to 2 plus or even 3 plus. Using the criteria described, reactions of 2 plus or greater offer evidence of disturbed liver function. Hanger believes that a positive test is an indication of active parenchymal disease, and in support of this may be cited the high proportion of patients suffering

from acute viral hepatitis who exhibit positive cephalin-cholesterol flocculation. Positive tests occur within a few days of onset of clinical illness, frequently before jaundice or significant elevation of bilirubin appears. Thus it offers valuable support for a diagnosis of viral hepatitis, particularly in hepatitis without jaundice. However, failure of the cephalin-cholesterol test to become abnormal does not exclude the diagnosis. Neefe, Gambescia, Gardner, and Knowlton (1950) found that 20 per cent of a large group of patients ill of viral hepatitis gave negative tests. Abnormal cephalin-cholesterol flocculation readings may or may not persist into the stage of recovery. In this respect it differs from the thymol test, which tends to remain positive for a considerable time.

A high proportion of cirrhotic patients show positive tests. Positive tests are prevalent in a variety of diseases such as malaria, pneumonia, infectious mononucleosis, and others causing damage to the liver. Biliary obstruction of short duration is characterized by negative cephalin cholesterol flocculations, and it may be used along with other observations to aid in determining whether jaundice is due to causes that may require surgical intervention. Inflammatory disease of the bile ducts usually is accompanied by positive tests.

The mechanism producing flocculation of the cephalin-cholesterol reagent appears to differ somewhat from that of the thymol test. Moore, Pierson, Hanger, and Moore (1945) showed that the change in serum responsible was associated primarily with the albumin fraction. Addition of normal serum albumin to serum giving a positive test may alter the response to negative; hence care must be taken to avoid collection of serum for testing during the 12 to 24 hours following administration of albumin parenterally.

A number of attempts have been made to establish a chemical difference between albumin of sera giving positive reactions with cephalin-cholesterol reagent and that of normal sera. Charlwood (1954) found no indication of a significant difference in albumins separated electrophoretically from sera of patients with liver disease. Hanger (1947) finds the inhibiting action of albumin to be variable. Martin (1951) found that albumin from serum of patients with hepatitis had less effect on the thymol turbidity.

A relationship of α_1-globulin to the flocculation of serum proteins by cephalin-cholesterol reagent has been postulated. Grassmann *et al.* (1951) describe a patient with cirrhosis whose α_1-globulin was elevated and attribute the negative cephalin-cholesterol test to this.

Acid-Precipitable Globulin Turbidity Test

A new turbidity test, the acid-precipitable globulin (APG) turbidity test, has been described by Greenspan (1955). Serum is added to an acetate buffer of low ionic strength adjusted to *p*H 4.42. He reports that treatment of sera of patients with obstructive or inflammatory lesions of the biliary tract leads to markedly increased turbidity readings. In hepatitis, the response was similar to that in normal persons but in cirrhosis a tendency toward low readings existed. Alpha$_2$- plus β-globulins are measured by this reagent.

Abnormalities in Blood Clotting in Liver Disease

These are another manifestation of the disturbances in protein metabolism, although associated disturbances in lipid metabolism also may be important. It is now known that the delayed clotting of blood of many patients with liver disease is the result of a combination of defects. Besides the diminished prothrombin that commonly is found in both acute and chronic liver disease, deficiency of accelerator globulin (Ac-globulin, Factor V, labile factor, proaccelerin, etc.), and probably of other factors occurs. Alexander and Goldstein (1950) report not only a lowering of labile factor but also of serum prothrombin conversion factor. Fibrinogen concentration may be low, as already mentioned. Fibrinolysin activity of plasma may be increased.

Selman (1952) has reported on the occurrence of low antithrombin titers in serum hepatitis. A clotting factor, thrombin accelerator, in cirrhosis is defined by Ratnoff (1953).

Hepatectomy causes a decrease in fibrinogen, "thrombogen," and antifibrinolysin (Nolf and Adant, 1951) and in prothrombin, cothromboplastin, labile factor, as well as fibrinogen (Mann, Shonzo, and Mann, 1951). The complexity of the disturbance of clotting function in liver disease is apparent in recent reviews of this topic (Harrington *et al.* 1950; Stefanini, 1953; Alexander, 1955). Alexander (1955) points out that in addition to the defective clotting mechanism, vascular abnormalities and thrombocytopenia contribute to deficiencies in clotting in patients with liver disease. He points out that the multiple abnormalities may have a more than additive effects.

The depletion of prothrombin in biliary obstruction is related to impaired vitamin K absorption. This in turn is due to the failure of bile to reach the intestine in sufficient amounts to maintain absorption of fats and fat-soluble vitamins. Perhaps a similar mechanism contributes to the lowered prothrombin activity in parenchymal liver disease. Vita-

min K administered parenterally in large amounts usually has little effect on the lowered plasma prothrombin activity of patients suffering from parenchymal liver disease. On the other hand, it corrects this defect in patients with biliary obstruction, provided liver damage is not excessive.

Methods for Evaluation of Clotting Function

These include studies of plasma prothrombin activity, accelerator factor, fibrinolysin, and, at times, fibrinogen. Numerous technics for prothrombin and fibrinogen measurements are described in the literature.

Estimation of prothrombin activity is an indispensable preliminary to surgical operations on patients with disease of biliary tract, liver, or small intestine, and to needle biopsy of the liver. It is used also to evaluate the response to vitamin K administered to jaundiced patients. A substantial rise in prothrombin activity to values that are within or approaching the normal range suggests that liver function is not greatly impaired: thus the jaundice may be presumed to originate from a lesion of the biliary tract (Lord and Andrus, 1941; Shapiro and Richards, 1945). One of the important shortcomings of this method is the lack of precision of prothrombin measurements, as ordinarily measured, in the 30 to 100 per cent range.

Many patients with parenchymatous liver disease maintain prothrombin activities within normal limits throughout the course of their illness [according to Stefanini (1949), roughly half]. This is true especially if liver involvement is of minimal or moderate severity. For this reason the measurement of prothrombin activity is not as efficient for detection of liver damage as are a number of the other procedures described in this review. However, an occasional patient will have a significantly lowered prothrombin activity due to liver disease when other simple tests, including the serum-bilirubin concentration and flocculation and turbidity group, fail to give an abnormal response.

Impairment of Energy Production and Storage

Liver disease is associated with increased concentrations in blood and urine of the di- and tricarboxyl acids that constitute pathways by which foods are converted into energy. Considerable attention has been given, by Scandinavian workers especially, to the elevation of plasma citrate in patients suffering from liver disease. These studies have been reviewed by Sjöström (1936). Bunker *et al.* (1955) report that patients with liver

disease are susceptible to citrate intoxication when transfused with large volumes of citrated blood. Marked elevation of serum citrate occurs as a result of impaired ability to utilize this substance.

Others have found that lactate (Snell and Roth, 1932), pyruvate (Amatuzio and Nesbitt, 1950), alpha-ketoglutarate (Seligson, McCormick, and Sborov, 1952) and succinate (Emmrich, 1948) concentrations also are elevated. These findings suggest the occurrence of a general increase in concentration of such substances. The occurrence of such an increase in turn suggests that the efficiency with which these transformations are being made is impaired in the presence of severe parenchymal liver disease. Thus, the supply of energy available to the cells is decreased. The effects on cellular metabolism would be widespread and important, and would be manifested in decreased rates of synthesis of the various products made by the liver cells as well as impairment of regulatory and other functions. Recant (1954) recently described a defect in ketone metabolism in patients with cirrhosis. This was characterized by average blood ketone concentrations of 0.94 mg./100 ml. in cirrhotics compared with 2.28 mg./100 ml. in normal persons.

Saltzman and Caraway (1953) have described a method for measurement of the efficiency of an oxidation, the conversion of cinnamic to benzoic acids, in vivo. They find a reduced rate in patients with liver disease.

Studies of certain components of the phosphate cycle have been made. Helve (1946) reports no change in major fractions of the blood cell phosphate. However, it is difficult to establish impairment in functioning of the phosphate cycle in vivo by analysis of concentrations because its efficiency is a function of the rate of turnover rather than of concentration. Smith, Ettinger, and Seligson (1953) state that serum inorganic phosphate decreased following injection of glucose and fructose to the same extent in patients with liver disease as in normal persons, while in diabetes mellitus little change in its concentration occurred. The marked rise in serum creatine concentration and increase in creatine excretion previously described suggests that phosphocreatine is being hydrolyzed and presumably not rapidly reformed. As a result, stores of high energy phosphate are being depleted.

Waterlow (1953) has studied the enzyme activity of samples of liver removed by punch biopsy from infants suffering from liver disease of nutritional origin. His material included specimens from patients with kwashiorkor or the closely related nutritional liver disease of Jamaican infants, as well as samples obtained from control subjects. He found a

wide variation in susceptibility of various respiratory enzymes to severe malnutrition and associated liver damage. His work, and that of others using animals, emphasizes the importance of low protein intake in causing depletion of certain enzymes.

Measurement of the activities in serum of several enzymes which are liberated from the liver acutely damaged by various agents appears to offer new leads for study of liver disease. Aldolase, the enzyme splitting fructose-1,6-diphosphate into the triosephosphates, has been studied by Bruns and Puls (1954) and by Cook and Dounce (1954). Striking increases in activity were observed in sera of patients suffering from acute hepatitis. On the other hand, little increase over normal activities was found in cirrhosis, latent hepatitis, or biliary obstruction. Phosphohexoseisomerase, an enzyme transforming glucose-6-phosphate to fructose-6-phosphate, behaved in the same manner as aldolase (Bruns and Jacob, 1954; Bruns and Hinsberg, 1954). Wroblewski and LaDue (1954) report that the activity of glutamic-oxaloacetic transaminase rises in serum in patients with severe liver damage. None of these changes are specific indications of liver involvement, for the activity of serum aldolase is greatly increased in activity in muscular dystrophy (Dreyfus *et al.*, 1954; Jacob and Neuhaus, 1954) and glutamic-oxaloacetic transaminase in coronary occlusion (LaDue *et al.*, 1954). Methods for estimating these enzymes may be found in the articles listed. A spectrophotometric method for glutamic-oxaloacetic transaminase is described by Karmen *et al.* (1955).

The relationship between serum and hepatic enzyme activities has been studied experimentally by Koch-Weser *et al.* (1951). They found that in chronic liver damage produced by a diet low in protein and by chemicals in small amounts, serum and hepatic enzymes were lowered in roughly parallel manner. During regeneration of liver when cellular activity was vigorous, augmented activity of serum enzymes was observed. Acutely damaged cells permitted escape of enzymes from liver tissue. A tendency toward high values in serum for 24 to 48 hours was followed by subnormal activities.

Hormone Metabolism

Chronic liver disease is often accompanied by enlargement of the breasts in males and other evidences of altered sex hormone metabolism. A number of studies have been made of the excretion of various hormones in liver disease. Lloyd and Williams (1948) observed that excretion of 17-ketosteroids, androgens, and follicle-stimulating hormone was

diminished in cirrhosis of the liver. Dohan *et al.* (1952) found a significant increase in estrogen excretion in cirrhotic male patients. This was especially marked if they showed signs of gynecomastia. Spider nevi were associated with increased estriol excretion. 17-Ketosteroid excretion was significantly decreased. A decrease in gonadotrophin (FSH) was associated with testicular atrophy.

Peterson *et al.* (1954) surveyed steroid excretion patterns in several different diseases of liver and in patients with biliary obstruction. Some patients with hepatitis had normal or even increased 17-ketosteroid excretion, although the prevailing tendency in hepatitis, as in cirrhosis, was toward low values. Corticosteroid excretion was found to be increased in many patients with cirrhosis and the suggestion is made that conversion of these substances to ketosteroids is impaired in liver disease. Bongiovanni *et al.* (1954) report a decreased rate of conjugation of adrenal corticoids in cirrhosis. Brown *et al.* (1954) found the rate of disappearance of infused hydrocortisone to be inversely proportional to liver damage as measured by bromsulfalein retention. Patients with liver disease had normal fasting 17-hydroxycorticosteroid concentrations in plasma but excreted less in urine, evidence perhaps of a homeostatic adjustment. Miller and Axelrod (1954) found that cortisone perfused through rat livers was metabolized more slowly after the liver was made cirrhotic. Even more interesting is the finding that β-hydroxyl derivatives were formed in the cirrhotic but not in the normal liver. Schedl *et al.* (1953) reported differences in type of hormone excreted by patients with cirrhosis.

The liver produces enzymes that oxidize testosterone and related steroids. Injected testosterone is inactivated less rapidly in hepatectomized dogs than in intact dogs (West, 1951). Even mild liver damage decreased the proportion of injected testosterones excreted as 17-ketosteroid by patients (West *et al.*, 1951). Cantarow and associates (1951) found that the conjugation of injected testosterone was decreased in patients with liver disease. Adrenocorticotrophic hormone is inactivated by liver tissue (Eversole and Giere, 1951; Geschwind and Li, 1952).

The association of liver damage with hyperthyroidism was studied by many workers in the 1930's. A review by McIver (1942) summarizes the early findings. Maclagan and Rundle (1952) have confirmed earlier work by Althausen and Weaver (1938) demonstrating decreased galactose tolerance. Changes in rate of galactose absorption from the gastrointestinal tract may be important. Bartels (1938) found that 68 of 78 patients suffering from hyperthyroidism excreted less than 3 Gm. of

hippuric acid, the lower limit of excretion found in persons with normal liver function. A recent paper on liver function in hyperthyroidism is that of Lamberg and Gordin (1954).

Disturbances of Water and Electrolyte Balance

Retention, loss, or maldistribution of electrolytes and the resulting disturbances of water metabolism are among the most important and troublesome disorders encountered in patients with liver disease. These are caused in part by impairment of certain functions of the liver related to water and salt metabolism. Impaired synthesis of plasma albumin, depletion of intracellular proteins and electrolytes, disturbed metabolism of the hormones of the pituitary and adrenal as described in a previous section, and interference with the flow of blood through the portal system are some of the numerous derangements that contribute to alterations in salt and water equilibria in liver disease [*see* Strub, Talso, and Kirsner (1955)]. Failure of the liver often is accompanied by impaired function of the kidney, which adds to the gravity of the situation.

Salt and water retention is one of the characteristic complications of Laennec's cirrhosis. Its causes are complex. Mechanical factors such as increased pressure in the portal venous system are important. The resulting alterations in renal hemodynamics impair the efficiency of renal function. The lowered serum albumin concentration, while favorable to glomerular filtration, also favors transudation from the capillaries and thus accumulation of water in the tissues. Hormonal factors are known to be important. Shorr *et al.* (1951) have described a vasodepressor substance, ferritin (or VDM), whose concentration in the body fluids, they claim, is regulated in large measure by the liver. In addition to ferritin, it is possible that the antidiuretic hormone of the posterior pituitary contributes to the oliguria and hyposthenuria that precedes or accompanies salt and water retention. Still other factors may be involved. Increased sodium-retaining activity was found in the urine of patients with cirrhosis (Chart and Shipley, 1953). Luetscher and Johnson (1954) attribute this to the presence of increased quantities of aldosterone.

It is possible that some of the same factors that cause sodium and water retention in nephrosis are operating in cirrhosis. Conditions favoring the loss of cellular protein and potassium, considered by Metcoff *et al.* (1954) to be of primary causative importance in salt and water retention in nephrosis, exist also in cirrhosis. Plasma volume generally is increased in cirrhosis (Bateman *et al.*, 1949; Gilder *et al.*, 1954). Patients suffering from cirrhosis have diminished ability to excrete water administered in

the course of a water-loading test. This may be decreased to one third of the control value in the presence of ascites (Ralli *et al.*, 1951). A reversal of the diurnal rhythm of water and sodium excretion has been described by Popper and Rosenbaum (1952).

Rigorous restriction of sodium intake appears to be the most effective method of preventing accumulation of salt and water in excessive amounts by the cirrhotic patient (Eisenmenger *et al.*, 1949; Gabuzda *et al.*, 1954). The patient on such a regimen, in turn, requires regular and frequent chemical study because of the threat of the salt-depletion syndrome. The hazard of the latter is greatest when large amounts of fluid, 2–10 L. or more, are removed by paracentesis. If the patient subsequently dilutes the remaining extracellular fluid by consumption of water, sodium concentrations may fall sufficiently to bring about the abnormalities in blood flow and blood supply to the tissues that characterize the low-salt syndrome (Nelson, Rosenbaum, Strauss, 1951; Holly and McLester, 1951). Serum-sodium concentrations as low as 110 mEq./L. have been observed. Circulatory failure in such circumstances may lead to coma and death.

Artman and Wise (1953) have stressed the importance of potassium depletion in liver failure. Low fluid intake, vomiting, urinary losses, diarrhea, and administration of glucose are listed as contributing causes. They found potassium deficiency to be critical in many patients in hepatic coma. Aikawa *et al.* (1953) found lowered exchangeable potassium in cirrhosis with a deficit in the cells that was refractory to therapy [*see also* Amatuzio *et al.* (1952)].

Elevation of serum potassium also must be guarded against, especially in those patients whose liver disease is accompanied by marked impairment of kidney function.

Stutzman and Amatuzio (1953) called attention to lowered serum magnesium concentrations in portal cirrhosis. Studies of serum magnesium recently made in the writer's laboratory in a patient in hepatic coma have demonstrated a low value of 0.39 mEq./L.

Serum Iron in Liver Disease

Serum-iron concentrations are markedly affected by liver disease. In hepatitis, serum iron rises to 2 or 3 times the highest concentration ordinarily found in health. (Peterson, 1952; Matassarin and Delp, 1952). Low concentrations occur in serum of some cirrhotic patients (Howard, 1950).

Serum iron remains within normal limits in most patients with biliary obstruction. Since elevated values are prevalent in the presence of liver

damage, one might expect that measurement of serum iron would aid in differentiation of these two causes of jaundice. Recently, Christian (1954) and Landau (1954) have advocated its use for this purpose. The consensus of those who have attempted such an application is that it has relatively little value.

Serum iron determinations and especially determinations of serum iron-binding capacity, are of special interest in hemochromatosis. In this condition, accumulations of exceptionally large amounts of iron in the body lead to its deposition in liver, skin, and other tissues. The architecture of the liver is distorted as in cirrhosis. Fibrosis of the pancreas with diabetes mellitus is characteristic.

Serum iron concentrations are elevated in hemochromatosis, although not necessarily to concentrations higher than those occurring in health or in hepatitis. However, the measurement of iron-binding capacity in hemochromatosis consistently shows that the serum is almost completely saturated with iron. Gitlow, Beyers, and Colmore (1952) cite saturation values of 74 to 99 per cent as compared with 14 to 69 per cent in cirrhosis and 28 to 58 per cent in normal individuals. In hepatitis and all other conditions, at least a third of the total iron-binding capacity remains unsaturated.

Kleckner *et al.* (1955) have assessed the procedures used for diagnosis of hemochromatosis. In contrast to most observers, they state that serum iron-binding capacity may be saturated also in acute hepatitis, portal cirrhosis, transfusion hemosiderosis, aplastic anemias, etc., as well as in hemochromatosis. Liver biopsy, they state, is the best diagnostic criterion. Hemochromatosis should be distinguished from hemosiderosis, a condition characterized by excessive accumulation of iron in the tissues, largely as hemosiderin, as a result of malnutrition or of excessive iron administered either as blood or therapeutically as iron compounds. It lacks the pathologic changes in liver and pancreas characteristic of hemochromatosis. Klein *et al.* (1955) were unable to establish differences between hemochromatosis and hemosiderosis by analysis of iron in tissues. Kleckner *et al.* (1955) state that a patient with hemochromatosis may have a total iron content of 58 Gm. as compared with a normal of 4 Gm. Extremely high serum iron concentrations may occur in some patients with hemochromatosis (Howard *et al.*, 1954).

Methods for Study of Iron and Iron-Binding Capacity

Among several practical methods for serum iron are those of Barkan and Walker (1940) and of Kitzes *et al.* (1944). Iron-binding capacity can

be determined by the method of Cartwright and Wintrobe (1949) and of Ventura (1952).

Spectrophotometric iron-binding capacity measurements are unreliable if serum is icteric, hemolyzed, or lactescent. Peters and Apt (1955) have very recently described a new method based on the adsorption of ionic iron on a resin.

Copper and Diseases of the Liver

Wilson's disease (hepatolenticular degeneration) is associated with abnormalities in metabolism of copper (Glazebrook, 1945; Denny-Brown and Porter, 1951). The copper content of brain, liver, and urine is increased. After treatment with BAL (2,3 dimercaptopropanol) urine-copper concentration decreases. Bearn and Kunkel (1954a) report subnormal serum copper concentrations in 16 of 18 patients with Wilson's disease. The output of copper in urine was decreased also in these patients. Ceruloplasmin, a copper containing protein of serum, described by Holmberg and Laurell (1947), is present in lower concentrations in Wilson's disease than in healthy persons (Scheinberg and Gitlin, 1952; Bearn and Kunkel, 1954a). In Wilson's disease, Bearn and Kunkel (1954b) observed that Cu^{64} administered orally was immediately taken up by serum albumin. However, the transfer to an α_1-globulin that occurred in healthy persons and in patients failed to proceed in patients with Wilson's disease. In Laennec's cirrhosis, serum copper tends to be elevated (Bearn, 1953). The abnormal copper metabolism of Wilson's disease has been reviewed recently by Denny-Brown (1953).

Severe and persistent aminoaciduria is characteristic of Wilson's disease (Uzman and Denny-Brown, 1948; Stein, Bearn, and Moore, 1954).

There is sufficient liver involvement in Wilson's disease to produce abnormalities in the liver-function tests in a high proportion of patients suffering from this condition (Franklin and Bauman, 1953). However clinical and laboratory evidence of liver disease may be lacking in some.

Detoxification Reactions of the Liver

Numerous studies have demonstrated that in liver disease various reactions associated with detoxification are impaired. The best known example is the synthesis of hippuric acid following administration of benzoic acid, extensively studied by Quick (1940). Whereas at least 70 per cent of injected benzoic acid is excreted as hippuric acid within 1 hour by healthy individuals, less than this amount is excreted by pa-

tients with liver damage. Quick believes that the factor limiting hippuric-acid synthesis in liver disease is inability to mobilize glycine; however, this has been disputed (Voight, 1951). Voight compared the excretion of hippuric acid by patients with liver disease before and after they had received glycine. In many patients the rise in output was insignificant; moreover, the proportion excreted during the first 2 hours was not increased. A revived interest in hippuric-acid excretion tests in Germany seems to have resulted from the observations of Voight (1951) and Hartmann (1951) that hourly collection of urine after 6 Gm. of sodium benzoate gives a better clue regarding liver function than does the measurement of total excretion. Normally most of the excretion occurs during the first 2 hours. In hepatitis and cirrhosis the excretion is diminished and the greater percentage appears during the third and fourth hours. By applying this technic, Bogenhard (1954) found 90 to 95 per cent positive tests in hepatitis and 74–79 per cent positive in suspected hepatitis. Quick had originally recommended hourly collections, but this practice was abandoned because of emphasis on total output and also because of the extra labor required.

The conjugation of benzoic acid with glycine has been demonstrated in liver homogenates by Borsook and Dubnoff (1947). It occurs also in kidney, spleen, and probably other tissues. Benzoyl-coenzyme A appears to be an intermediate in hippurate synthesis (Schachter and Taggart, 1953).

Deiss and Cohen (1950) have described a similar test in which para-aminobenzoate replaces benzoate, and the conjugation is evaluated by analysis of serum. The para-aminohippurate formed is estimated colorimetrically in a sample of blood collected 1 hour after the test dose.

An increased excretion of hippuric acid in "free anxiety," a condition of exaggerated foreboding or apprehension, was observed by Persky *et al.* (1950). Deiss and Musser (1950) described a similar effect upon para-aminohippurate synthesis. Musser *et al.* (1955) found that administration of glycine had little effect in augmenting synthesis of para-aminohippurate in the presence of free anxiety, in comparison with normal controls. They studied hyperthyroid patients also, and found that a lowered synthesis characteristic of this condition was greatly enhanced by glycine.

Conjugation of various substances with glucuronic acid also is impaired in liver disease (Wagreich *et al.*, 1941; Ottenberg *et al.*, 1943). Snapper and Saltzman (1949) have described a test of liver function that measures glucuronate conjugate excretion after administering

sodium cinnamate. Its application to the study of liver disease is evaluated by Sharnoff *et al.* (1951). Saltzman and Caraway (1953) have described a refinement of the cinnamic acid test in which blood cinnamic acid levels are measured.

Hartmann (1951) has evaluated the use of detoxification reactions other than that of benzoate for study of liver disease. Intramuscular injection of guaiacol followed by measurement of sulfate and sulfate-ester excretion showed decreased conjugating ability in hepatitis and cirrhosis, with normal values in cirrhosis. Glucuronate excretion appeared to offer less information. Increased outputs occurred in hepatitis. The xanthoproteic-acid reaction applied to serum gave increased values in hepatic precoma and coma. Salicylate or thiocyanate excretion tests were not useful for study of liver disease. Deamination of tyramine, a pressor amine, occurs in the liver (Hare, 1928) and it is thought that other substances of this type are similarly inactivated.

Methods for the Study of Detoxification Function

Measurement of hippuric acid excretion is most frequently used for this purpose. However, availability of more simple procedures for study of liver disease appears to have decreased the use of the hippuric-acid test. The technic of Quick (1940) may be used. Intravenous administration of hippuric acid is preferred. The conditions under which the test is done are important. The patient should ingest sufficient water to insure adequate urine flow (Machella, Helm, and Chornock, 1942). He must be physically and mentally relaxed.

Hippuric acid is readily excreted by the kidney but marked impairment of kidney function lowers the output. Body weight and sex should be taken into account in interpretation of results (Hepler and Gurley, 1942).

Zieve *et al.* (1950) include measurement of hippuric-acid synthesis in a combined test that also measures galactose utilization and bromsulfalein retention. Zieve and Hanson (1953) found that administration of benzoic acid on 3 consecutive days caused a marked increase in hippurate-forming ability of patients with liver disease. A higher proportion of the benzoate was conjugated on each successive day.

A chromatographic method for quantitative determination of hippuric acid described by Gaffney *et al.* (1954) requires only 10–100 μL. of urine. Kimbel (1955) has developed a micro method for para-aminohippurate in blood.

The para-aminohippurate test of Deiss and Cohen (1950) has the great advantage of avoiding the necessity for urine collections, a major

source of difficulty in the hippuric-acid test. Trial of this procedure is desirable. The same may be said of the new cinnamic acid test of Saltzman and Caraway (1953).

Serum Alkaline Phosphatase

Measurement of alkaline phosphatase activity of serum often assists in the differentiation of parenchymal liver disease from that due to obstruction and other lesions of the biliary tract. Biliary obstruction is characterized by an increase in phosphatase activity to two or more times the maximum found in healthy individuals (Roberts, 1933), and by the persistence of such high activities. Some increase occurs also in patients suffering from viral hepatitis; however, the rise is moderate and transitory. Toxic hepatitis caused by chemicals or drugs is usually accompanied by elevated phosphatase activities. Several reports indicate that chlorpromazine (thorazine) therapy may cause phosphatase activity and bilirubin concentration of serum to rise to the same extent as in biliary obstruction (Zatuchni and Miller, 1954; Loftus *et al.*, 1955). In cirrhosis of the Laennec type, no marked rise in alkaline phosphatase activity occurs ordinarily, but at times, high activities may be encountered.

The rise in phosphatase associated with obstruction of the bile ducts occurs regardless of the nature of the obstruction, whether due to calculus, stricture, or neoplasm. Elevation of serum alkaline phosphatase may provide one of the few clues to the presence of neoplastic growth in the liver (Rothman *et al.*, 1936; Flood *et al.*, 1937; Ricketts *et al.*, 1950; Shay and Siplet, 1954). Inflammatory disease of the bile ducts (cholangiolitic hepatitis) causes striking increases in serum alkaline phosphatase (Watson and Hoffbauer, 1946).

The chemical changes occurring in this condition are indistinguishable from those observed in many patients with extrahepatic biliary obstruction.

The cause of the increase in serum alkaline phosphatase activity occurring in patients suffering from biliary obstruction has provoked much discussion. The liver appears to be able to remove phosphatase from blood plasma and excrete it in the bile. However, hepatectomized animals show, at most, a moderate rise in phosphatase activity of serum (Freeman, 1951; Flock *et al.*, 1952). This suggests that failure of the liver to excrete phosphatase produced in other organs is responsible only to a limited extent. Histochemical studies show high concentrations of phosphatase in the cholangiolar epithelium (Burke, 1950) in cholangiolitic

hepatitis. Sherlock and Walshe (1947) found increased amounts in the hepatic cells and nuclei in hepatitis and in bile-duct obstruction. The amount in the sinusoidal walls also was found increased. Thus it appears possible that the excess phosphatase in plasma may have several sources. The predominant source may be the cholangiolar epithelium in biliary-tract disease and the liver parenchyma in hepatitis. Rosenthal and associates (1952) have shown that regenerating liver parenchyma is very rich in phosphatase. Roche and Sarles (1954) describe experiments that suggest that the liver regulates concentrations of alkaline phosphatase activities of serum.

Applications of phosphatase-activity measurements include not only the detection of involvement of the biliary tract but also the evaluation of the clinical course of patients with such lesions. Ulevitch *et al.* (1951) have pointed out that phosphatase activity and serum bilirubin may change independently and that phosphatase activity is the more sensitive indicator of change in degree of biliary obstruction.

Bile-Pigment Metabolism

Jaundice is such a conspicuous sign of liver damage or bile duct blockage that it has attracted more than its share of attention, often to the neglect of other and more important aspects of liver or biliary tract disease. A number of excellent reviews of bile pigment chemistry and metabolism have appeared within the last few years (Watson, 1946; Lemberg and Legge, 1949; Gray, 1953; Lathe, 1954; Gray, 1954). With the evidence provided by McNee (1913) in Aschoff's laboratory, Whipple and Hooper (1913), and Mann (1921) demonstrated that the major site of bilirubin formation from hemoglobin was the Kupffer cells of the reticuloendothelial system throughout the body. [*See* Lathe (1954) for the history of these developments.)

Certain peculiarities in the behavior of bilirubin suggest that it may exist in serum in two forms. These are readily differentiated by means of their rates of reaction with diazotized sulfanilic acid (Hijmans van den Bergh and Müller, 1916). A rapidly reacting form of bilirubin (direct or prompt reacting bilirubin) is considered to have been secreted into the bile by the parenchymal cells and subsequently to have returned to the blood stream because of disruption of the liver structure by parenchymal disease or because of biliary obstruction. This form of bilirubin (cholebilirubin) is distinguished from a second (hemobilirubin) considered to be in transport from extrahepatic sites of hemoglobin breakdown to the liver. The latter reacts with the diazo reagent slowly and yields addi-

tional color after alcohol, caffeine, or other catalysts are added to the reaction mixture.

All the bilirubin in serum is bound to protein, chiefly albumin, but it is possible that a portion is more firmly bound. Plasma protein fractionation by Cohn (1948) and his associates has yielded an alpha-globulin which, it is stated, binds bilirubin firmly and specifically. Martin (1949) has described its characteristics, among them a delaying action on the diazo reaction of bilirubin bound by it. Attempts to establish a structural change in the bilirubin molecule that might provide a basis for the observed difference have led to negative or equivocal results. However, Najjar (1952) recently described the preparation from serum of two bilirubins differing in crystal form and solubility and in their reactivity with the diazo reagent. Other factors influencing the rate of reaction include the concentration of bilirubin and its degree of dispersion. There is reason to believe that the serum lipid may be involved. For a review of the various hypotheses concerning the diazo reaction of serum bilirubin the reader is referred to Gray (1953) Chapter VIII, also Lathe (1954).

Cole and Lathe (1953) succeeded in separating serum bilirubin into two components by means of reverse phase chromatography using silicone-treated kieselguhr as adsorbent. A rapidly moving type is more soluble in water and gives the direct diazo reaction (without addition of alcohol). The other is more soluble in organic solvents and reacts only in presence of alcohol. The latter is thought to be bilirubin. The nature of the fast-moving component is not established, but it predominates in serum in obstructive jaundice and in bile. In hemolytic jaundice the slower component predominates. No protein was present in the extracts of serum studied, hence Cole and Lathe conclude that differing degrees of association of bilirubin with protein cannot be responsible for the differences in behavior of various serums with the diazo reagent. The presence of several pigments in bile, some of which do not react with the diazo reagent, was demonstrated.

The direct reacting pigment found in serum of patients with obstructive jaundice and in bile was separated in turn, into two pigments by Cole, Lathe, and Billing (1954), each giving the direct reaction. They differ from bilirubin in their much greater solubilities in water, and in their absorption spectra. The latter have a maximum at 419 mμ in contrast to bilirubin which had a maximum at 454 mμ.

Lathe (1954) believes that bilirubin is first converted to Pigment I (see below) by the liver, since it appears in blood in substantial amounts after injection of bilirubin whereas there is little Pigment II. The latter, however, is more abundant in bile.

Sera obtained from patients ill with hemolytic anemias or pernicious anemia when tested with the diazo reagent produce relatively little color in 1 minute compared to that produced after addition of methanol. This is true also after administration of aged or incompatible blood and the serum of newborn. On the other hand, approximately 50 per cent of the total color appears in 1 minute when the sera of patients with biliary obstruction or moderate to severe parenchymal damage are tested. Kernicterus, an intense pigmentation of the basal ganglia associated with jaundice of the brain, occurs occasionally in newborn infants. Some impairment of bilirubin excretion during the first days of life appears to be a general occurrence in all newborn and if rapid blood destruction occurs at this time, such as that produced by erythroblastosis due to development of anti-Rh agglutinin in the mother, very high serum-bilirubin concentrations may occur. Either because of this or because of associated changes in the brain, permanent injury to the brain or death may result. The nature and significance of the brain pigmentation is discussed by Claireaux *et al.* (1953), Vogel (1953), and Day (1954). The attending pediatrician may be guided in his treatment of the newborn by the height of the serum bilirubin, in relation to other factors, and his decisions will be influenced also by the rate of increase in its postnatal concentration. Replacement of the infant's blood by means of exchange transfusion is a commonly accepted therapeutic approach.

The need for bilirubin determinations in these circumstances is such that the laboratory should be prepared to do them immediately. A series of measurements usually are required.

The serum bile-pigment concentration of normal 3629-Gm. (8-lb.) babies is usually less than 3–5 mg. per 100 ml., according to Lathe (1954); that of 1814-Gm. (4-lb.) babies is much higher and may rise to 10 to 15 mg. per 100 ml.

Another abnormality of bile-pigment metabolism has been described recently by Dubin and Johnson (1954a and b). Its symptoms are chronic jaundice, abdominal pain, and fatigue. The outstanding feature is the presence of large amounts of an unidentified granular brown pigment in the liver.

Methods for Measurement of Concentration of Bilirubin in Serum

Methods for measurement of bilirubin concentration of serum include the direct comparison of the yellow color of diluted serum with that of 0.01% potassium dichromate, the "icterus index" of Meulengracht (1921). This crude method is remarkable only because it serves so much better than one would anticipate in view of the many sources of error

to which it is susceptible. Henry *et al.* (1953) have described methods for avoiding some of the errors. It is quite unreliable unless serum bilirubin concentrations are elevated. Spectrophotometric measurement of bilirubin is feasible provided appropriate corrections are made for heme pigments and opalescence of the serum. However, the diazo reagent is by far the most generally used.

There has been confusion about the mechanism of the reaction of bilirubin with this reagent which Lathe (1954) has helped to clarify. He cites the work of Fischer and Haberland (1935) as demonstrating that the bilirubin molecule is split into two dipyrroles, neo- and isoneoxanthobilirubinic acid. In this way, the alpha position (adjacent to the nitrogen in the pyrrole ring) of one of the dipyrroles becomes available for coupling to the diazo reagent. The other dipyrrole would not react because the methylene group of the bridge would remain as hydroxymethyl. Presumably some of the bilirubin would split on one side and some on the other side of the methylene carbon. In this way a mixture of two diazo pigments—one the diazo derivative of neo- and the other of isoneoxanthobilirubinic acid—is formed. However, each molecule of bilirubin gives rise to only one diazo derivative. The remaining "half" is present as an unreactive alcohol.

Evaluation of the speed of the reaction of bilirubin with the diazo reagent, formerly judged by the eye, has been replaced by photometric measurement of the color formed 30 minutes (Malloy and Evelyn, 1937) or 1 minute (Ducci and Watson, 1945) after adding the reagent. Billing (1954) found a "reasonable correlation between the percentage of direct-reacting pigments found chromatographically and that obtained by the Malloy and Evelyn method at 30 minutes." However, the method of Ducci and Watson has proven to be the more useful clinically. Kingsley *et al.* (1953) have described the technic of the latter with an improved method for standardization. Shinowara (1954) has described the determination of bilirubin and hemoglobin with the aid of the spectrophotometer. Moreland *et al.* (1950) substitute sulfanilamide for sulfanilic acid in the diazo reaction.

The factors affecting serum bilirubin determinations have been discussed by Proffitt and Morrison (1949). *p*H and nitrite concentration are critical. White and Duncan (1952) report that bilirubin added to serum gives less color than it does in chloroform-ethanol solution, and that Beer's law is not obeyed when determinations are done by the Malloy and Evelyn method (1936) if the concentration exceeds 15 mg. per 100 ml.

A micro technic for measurement of serum bilirubin has been described by Hsia *et al.* (1952).

Berman *et al.* (1954) have discussed the relationship of xanthochromia of cerebrospinal fluid to serum bilirubin and have described a method for measurement of bilirubin in cerebrospinal fluid. [*See also* Amatuzio, Weber, and Nesbitt (1953).]

Interpretation

Total serum bilirubin of 95 per cent of healthy individuals is below 1.10 mg. per 100 ml. of serum. Occasionally persons who appear to be in good health will have concentrations as high as 1.50 mg./100 ml.

The direct reacting, "1-minute" bilirubin reading estimates bilirubin reacting with the Ehrlich diazo reagent promptly in the absence of ethanol, methanol, or other catalyst. This fraction represents 10 to 20 per cent of the total bilirubin of normal serum. In 95 per cent of healthy individuals it does not exceed 0.18 mg./100 ml. and in 99 per cent is below 0.25 mg./100 ml. In the presence of hyperbilirubinemia due to any cause other than increased hemolysis, the 1-minute direct reading increases to approximately 50 per cent of the total, and ranges from 30 to 70 per cent. The direct bilirubin may be elevated in serum of some patients whose serum total bilirubin is within normal limits, and it is somewhat superior to the latter for detection of liver involvement. When hemolysis occurs with severity sufficient to cause hyperbilirubinemia, the total serum bilirubin consistently is elevated to a greater degree than is the prompt reacting portion. However, the behavior of the serum bilirubin in jaundice caused by biliary obstruction does not differ from that occurring in jaundice caused by injury to the hepatic parenchymal cells, at least not to an extent that enables conclusions to be reached in a given patient regarding the etiology of the jaundice. Measurement of the rate of the diazo reaction of bilirubin adds little of value for differential diagnosis because hemolytic jaundice, the one condition that it distinguishes generally can be recognized by other more specific findings. The significance of serum-bilirubin determinations with special reference to the 1-minute reading has been discussed by Zieve *et al.* (1951) and Klatskin and Drill (1950).

Studies of total serum bilirubin are useful for following the clinical course of a patient. Rising serum-bilirubin concentrations in general have unfavorable implications; falling values are characteristic of remission of liver disease or biliary obstruction. A stabilized serum-bilirubin concentration is considered a highly desirable prerequisite to operation

for relief of biliary obstruction. ACTH and cortisone given to patients with increased serum bilirubin cause a marked decrease in bilirubin concentration of serum.

Tests for Bilirubin in Urine

Many of the procedures widely used for detection of bilirubin in urine are not sufficiently sensitive to be useful for detection of early hepatitis or moderate liver damage. Foord and Baisinger (1940) compared many of the commonly used procedures and found that the Harrison-Fouchet method as described by Godfried (1934) was one of the most dependable, and the experience of many workers has confirmed the value of this procedure. Several modifications of this method in which the reagents are applied to paper strips (Watson and Hawkinson, 1946) or to tablets of plaster of Paris have proven to be convenient although less sensitive than the original Harrison-Fouchet method. The latter concentrates the bilirubin in urine by adsorption on freshly precipitated barium phosphate and sulfate. The blue-green color produced by treating the precipitate with acid ferric chloride is quite specific for bilirubin and closely related pigments. Urobilinogen or urobilin do not react.

Other methods for testing urine for bilirubin include the methylene blue test which depends upon the extinction by additional methylene blue of the green color produced by interaction of bilirubin and methylene blue (Reinhold and Fowler, 1947). This test is easily applied, but it measures also other yellow pigments that may be present. It gives a roughly quantitative estimate of bilirubin concentration.

Recently, Free and Free (1953) described a rapid and sensitive diazo test for bilirubin which appears to compare favorably with the Harrison-Fouchet method and which may prove to be superior (Klatskin and Bungards, 1953; Sobotka *et al.*, 1953; Giordano and Winstead, 1953; Tallach and Sherlock, 1954).

Bilirubin is present in the urine of healthy persons in concentrations so low that it is not detected by ordinary methods. Positive tests for bilirubin in urine thus indicate the existence of liver damage or biliary obstruction. They occur, at times, before serum bilirubin is elevated. It is for this reason that urine bilirubin tests are useful for the detection of acute viral hepatitis in its early stages (Neefe *et al.*, 1944; Swift *et al.*, 1950). Later in this disease, urine bilirubin tests may become negative while the disease is still active and serum-bilirubin concentrations remain above normal limits. The means by which bilirubin passes the renal barrier is far from clear, since it is firmly bound to protein in serum and

nondiffusible or only slightly so. Some may enter the urine combined with protein which is a fairly regular constituent of urine of patients suffering from hepatitis. The remainder must be split from protein by a renal mechanism still unknown. An investigation of the relationship of the bilirubin in urine to the new pigments discovered by Cole *et al.* (1954) obviously is needed.

Shutkin and Caine (1955) report the detection of bilirubin in urines of 11 patients with subclinical liver disease out of 1000 routine tests of urines of hospital patients. The "Bilazo" test of Free and Free (1953) was used.

After the presence of liver disease has been established and if serum-bilirubin concentrations are being measured, urine bilirubin tests offer little useful information.

Urobilinogen in Urine and Feces

Urobilinogen is normally present in urine. It is formed by reduction of bilirubin by bacterial action in the cecum and colon. Urobilinogen is reabsorbed from the intestine and excreted mainly by way of the bile. Impaired excretory ability of the liver leads to an increase in its output in the urine. Thus, measurement of urobilinogen concentration in urine is a test of liver function. However, when the liver is damaged to such an extent that the secretion of bile is suppressed, no urobilinogen will be formed or detected. It is absent also if obstruction of the bile ducts prevents entry of bile into the intestine. Patients receiving (a) certain diets not conducive to maintenance of the usual bacterial flora, (b) antibiotics, or (c) other substances altering the bacterial flora of the intestine may excrete little or no urobilinogen in feces or urine.

Urobilin is formed by oxidation of urobilinogen. Whereas urobilinogen is colorless, urobilin is yellow. Urobilinogen is converted to urobilin on standing in the presence of oxygen, or by oxidizing agents. This change is delayed by protecting urine from light and air and by alkaline reactions. Urobilin may be reconverted to urobilinogen by treatment with ferrous sulfate and sodium hydroxide.

The presence of increased amounts of urobilinogen in urine is a characteristic finding in patients with disease of the parenchymal cells of the liver. At times it may offer the only conclusive evidence of the existence of liver disease. Changes in urine urobilinogen excretion are likely to occur independently of changes in other tests, and for this reason it is desirable to include it in a battery of such tests.

For more detailed information about urobilinogen and related pig-

ments, the recent review of Gray (1954) may be consulted. Watson *et al.* (1954) have studied the formation and interrelationship of urobilinogens by administration of bilirubin labeled with N^{15}. Under special conditions dextrorotatory urobilin (*d*-urobilin) may be formed in the intestine.

Two procedures are widely used for estimation of the urobilinogen excretion in urine. One is the simplified quantitative Ehrlich reaction of Watson, Schwartz, Sborov, and Bertie (1944), as modified by Watson and Hawkinson (1947). This method is designed for use as a routine or screening test and for most purposes is capable of supplying the information sought. However, it may be necessary to apply in special circumstances a method yielding more specific data, and for this reason the quantitative method of Schwartz, Sborov, and Watson (1944) is recommended. Balikov (1953) has improved the technic of the latter.

Interpretation

The excretion of urobilinogen in urine by healthy persons averages 0.64 mg. in 24 hours. Only 2.5 per cent excrete more than 1.56 mg. and only 0.5 per cent more than 2.1 mg. in 24 hours. Patients with disease of the liver may excrete amounts of urobilinogen greatly exceeding these limits —up to 100 mg. or more in a 24-hour urine collection—although values ranging from 2 to 10 mg. are more usual. However, the excretion may fall within normal limits in patients with severe liver damage owing to the small amount of bile pigment reaching the intestine of such patients. Thus high values may be encountered following the onset of jaundice, to be followed by a period in which the output is within normal limits. This may persist for only a day or two or may continue for 1 or more weeks. It, in turn, is followed by a second period of elevated excretions which gradually return to normal limits as the illness improves.

Urobilinogen (Stercobilinogen) in Feces

The urobilinogen of feces consists of several closely related compounds differing in minor details of structure. The principal one is stercobilinogen which is distinguished from urobilinogen by measurement of optical rotation. Stercobilinogen reacts with Ehrlich's aldehyde reagent and the procedures for its determination are similar to those described for urine. Measurement of stercobilinogen output provides useful information to the clinician concerning (1) the rate of hemoglobin destruction and (2) the completeness of obstruction of the bile ducts. Such information may contribute substantially to the correct evaluation of the patient with jaundice. Knowledge of the rate at which hemoglobin is destroyed is of great value also in study of anemias.

Interpretation

Healthy persons excrete 100 to 350 Ehrlich units per 100 Gm. of feces, or 40 to 280 mg. as stercobilinogen. As much as 2000 mg. may be excreted each day by patients suffering from hemolytic anemia. Transfusion of blood often is followed by an increased excretion of stercobilinogen in feces, and may obscure interpretation of the findings.

Obstruction of the bile ducts caused by neoplastic or other growths is characterized by complete or nearly complete interruption of the flow of bile into the intestine. The daily excretion of stercobilinogen by such patients is only 1 or 2 Ehrlich units (or milligrams) and infrequently exceeds 5 Ehrlich units. Obstruction caused by calculi interferes less with excretion of bile and the daily output of stercobilinogen in feces will exceed 10 Ehrlich units. It is important to keep in mind, however, that either parenchymal or cholangiolitic disease of the liver, if severe, may suppress secretion of bile or otherwise prevent its entry into the intestine. As a result, stercobilinogen outputs as low as those found in obstruction due to neoplastic growth may be encountered in hepatitis, cirrhosis, or cholangitis. The low outputs are generally transitory in the latter, in contrast to the persistently low values characteristic of complete mechanical obstruction.

Measurement of Excretory Capacity of the Liver

Bile acids and various other substances, among them a number of dyes, are taken up by the liver and rapidly secreted into the bile. In liver disease the rate of excretion may be lowered, and because of this the use of certain of these substances has been of great value in measurement of liver function. Disodium phenoltetrabromophthalein sulfonate (sulfobromophthalein, sodium; bromsulfalein), introduced by Rosenthal and White (1925), has proved to be superior to numerous other substances tested for this purpose.

The diversion of much of the blood that ordinarily would flow through the liver into collateral channels is an important cause for delayed removal of injected bromsulfalein from the blood stream. As already mentioned, such a diversion becomes a major factor in cirrhosis, but it may be significant also when the liver is injured by viral or chemical agents and in patients with heart failure. A second important factor is the decreased mass of functioning liver cells, and a third may be inability of damaged liver cells to transport the dye into the bile. It was believed for a long time that the Kupffer cells were mainly responsible for removal of bromsulfalein from the blood stream. Mendeloff (1949 a and b) compared the activities of hepatic polygonal cells in removing Rose Bengal, a dye

closely resembling bromsulfalein in its behavior, from the circulation. Fluorescence microscopy demonstrated no dye in Kupffer cells, while the polygonal cells contained large amounts. Brauer and Pessotti (1948) found that bromsulfalein was taken up by slices of liver but not by splenic or lymph node slices.

The belief, formerly widely held, that the reticuloendothelial cells were primarily responsible for removal of bromsulfalein from the circulation was based mainly on experiments in which India ink was injected into the blood stream. When bromsulfalein was injected following India ink, removal of the dye was delayed presumably because of blockade of the reticuloendothelial system by the carbon particles in the ink. However, Shore and Zilversmit (1953) and Zilversmit and Shore (1954) have demonstrated in convincing experiments that carbon suspensions are without effect on bromsulfalein excretion, whereas a carbon-free filtrate of India ink was sufficiently toxic to the liver to cause the delay in dye excretion.

Although up to 95 per cent of the bromsulfalein injected into healthy persons is rapidly removed from the circulation, only a small amount appears in the urine. The proportion excreted in the urine becomes much larger (Norcross *et al.*, 1951) in the presence of liver damage. Lattanzi (1950) reports 20 to 48 per cent excretion in 24 hours by patients suffering from liver disease.

Retention of bromsulfalein in patients with jaundice due to hepatitis or biliary obstruction may be prolonged for as much as 29 days (Giges, 1951). Patients with cirrhosis did not show this tendency. It is doubtful whether application of the bromsulfalein test to the study of jaundiced patients provides information not readily obtainable by other means. The prolonged retention of dye remaining in the circulation after previous tests and the complications introduced by the presence of bilirubin in measurement of dye retention make it essential to collect a control sample of blood before such a test is done. Zieve *et al.* (1951) have derived a correction to be applied to results of bromsulfalein tests if total bilirubin in serum exceeds 2 mg./100 ml.

A recent report by Brauer *et al.* (1955) suggests that the fate of bromsulfalein in the body may not be merely one of excretion in bile and urine. After bromsulfalein labeled with S^{35} was administered, the bile always contained more total S^{35} than the bromsulfalein. This apparently was not due to desulfonation.

The use of actual instead of ideal weight in calculating dosages of dye to be injected may cause erroneous results, especially in the presence of ascites, edema, or adiposity.

Rosenthal and White (1925) used 2.0 mg. per Kg. of body weight. The 5 mg. per Kg. dosage, however, has been adopted widely and provides a more sensitive and more precise test. The existence of an enterohepatic circulation of bromsulfalein (Lorber and Shay, 1952) does not appear to influence the results sufficiently to necessitate a return to the original dosage (Owen, 1951).

[Note: It is advisable to question the patient concerning sensitivities to drugs. Reactions to bromsulfalein are rare but a few individuals do not tolerate it, and it may have effects so severe as to constitute a medical emergency. A preliminary skin test may be done. Obviously, the injection of bromsulfalein should be done only by a physician or under his immediate supervision.]

The time at which the postinjection blood specimen is collected is not of great importance, provided that appropriate standards of normal for that time are used. Mateer *et al.* (1943) introduced the 45-minute sampling time and it has proved to be satisfactory. Calculation of the rate of disappearance of bromsulfalein by means of multiple specimens collected at 5- to 15-minute intervals after the injection has been advocated. The liver removes a constant fraction of the bromsulfalein remaining in the circulating blood during any time interval (Bradley, 1949) and by collecting a series of blood specimens the accuracy of the estimate of the bromsulfalein retained is improved. For a description of "clearance" studies of patients the reader is referred to papers by Deutsch (1941), Lewis (1948), Lavers *et al.* (1949), Goodman (1952), Goodman and Kingsley (1953), Neumayr *et al.* (1954), and Nadeau (1954).

Interpretation

Healthy persons after injection of 5 mg. of bromsulfalein per Kg. of body weight retain less than 10 per cent at 30 minutes and 7.0 per cent at 45 minutes. At 60 minutes, no dye is retained.

Bromsulfalein retention is generally accepted as the most sensitive and dependable among the laboratory procedures currently used to demonstrate involvement of the liver. It is especially helpful for evaluating suspicious or positive results obtained by means of flocculation tests in the absence of hyperbilirubinemia. The bromsulfalein test outscores all others in the proportion of positive tests found in Laennec's cirrhosis. It has been among the most useful for following recovery from viral hepatitis and for detecting residual liver damage from this disease. It is probably the only procedure capable of detecting fatty liver, although it cannot be depended upon to do so consistently.

Retention of bromsulfalein in the blood stream may occur because of

spasm of the sphincter of Oddi caused by morphine (Burnett, 1954), the mechanism being similar to that causing serum amylase to rise under similar conditions. Burnett (1954) reports that bromsulfalein was retained to an abnormal extent in 22 of 23 patients suffering from inflammatory disease of the gall bladder.

Miscellaneous Tests

Serum-Cholinesterase Activity

Serum-cholinesterase activity is usually lowered in the presence of atrophy of or damage to the liver parenchyma. Similar changes have been reported for serum esterase and lipase. Depression of serum cholinesterase activity closely follows changes in serum albumin concentrations. However, severe liver damage is not consistently accompanied by significantly lowered cholinesterase activity. Serial measurement of cholinesterase activity appears to provide a useful method for following the course of liver disease. It is of special value for study of the liver damage associated with deficient diets. Current articles dealing with its application to the study of liver disease include those of Mann *et al.* (1952), Fremont-Smith *et al.* (1952), Wilson *et al.* (1952), and Vorhaus and Kark (1953). Molander *et al.* (1954) have studied the effects of neoplastic disease involving the liver on serum-cholinesterase activity. Markedly depressed values were observed.

Serum cholinesterase may be measured conveniently by the method of Michel (1949) or by a photometric adaptation of it (Reinhold *et al.*, 1953).

Coproporphyrin in Urine

The output of coproporphyrin in urine rises markedly in patients suffering from various types of liver disease (Watson, Hawkinson, *et al.*, 1949; Watson, Sutherland, and Hawkinson, 1951). Measurement of coproporphyrin excretion provides a sensitive and valuable method for detecting liver damage that may escape detection by other methods. The excretion of coproporphyrin generally is within normal limits in patients who have biliary-tract lesions, but high values occur with sufficient frequency to impair its usefulness for differential diagnosis. Care must be used also to exclude other causes of increased porphyrin excretion; for example, various drugs and intoxicants. An idiopathic coproporphyrinuria has been described (Watson, Schwartz, *et al.*, 1949).

A simplified technic for determination of coproporphyrin has been described recently (Schwartz, Zieve, and Watson, 1951). The small amounts

of coproporphyrin in many samples of urine require the use of a highly sensitive fluorimeter for dependable measurements. The high cost of such instruments has been a deterrent to the wider use of porphyrin analysis. The improved methods now available give much higher results than those formerly used mainly because of the detection of porphyrin precursors in urine and their inclusion in the assay (Watson, Pimenta de Mello, *et al.*, 1951).

Watson and his associates (see references above) have shown that the type of coproporphyrin excreted is related to the cause of liver damage. Thus Type I coproporphyrin predominates in the urine of patients ill with viral hepatitis, whereas Type III predominates in cirrhosis of "alcoholic" origin.

Increased excretion of uroporphyrin in urine has been observed in patients with liver disease. Porphobilinogen excretion in urine also may be increased sufficiently to give a positive test in urine in the presence of liver disease (Watson *et al.*, 1954).

Combined Intravenous Bromsulfalein-Hippuric Acid-Galactose Test

Zieve, Hill, and Nesbitt (1950) have devised a procedure for administration of the three substances simultaneously. They find that simultaneous administration does not alter the behavior of the test substances.

Peptidase Activity of Human Serum

Fleisher and Butt (1953) have found tripeptidase activity to be increased in serum of patients with liver disease or with obstruction of the bile ducts. Bile shows marked tripeptidase activity. Hydrolysis of several dipeptides is decreased in liver disease, according to these authors.

Protein-Bound Iodine of Serum in Liver Disease

Kydd and Man (1951) found sharply increased concentrations of protein-bound iodine in serum during earlier stages of viral hepatitis. On the other hand, cirrhotic patients tended to have subnormal or low normal concentrations.

SELECTION OF PROCEDURES

The choice of methods to be applied in the study of a patient with disease of the liver or biliary tract will vary according to the type of information sought. The following outline shows the principal purposes for which chemical methods are applied together with a list of the procedures likely to prove useful.

Detection of subclinical liver disease in absence of jaundice: urine bilirubin, bromsulfalein retention, direct and total bilirubin, flocculation tests (cephalin-cholesterol flocculation, thymol turbidity and flocculation, zinc turbidity), urine urobilinogen, zone electrophoresis protein pattern.

Studies designed for the purpose of investigating the presence of residual viral hepatitis in veterans by Zieve *et al.* (1953) and Neefe *et al.* (1955) illustrate one problem to which this group of tests has been applied. Another is the testing of blood donors for the purpose of detecting certain carriers of viral hepatitis who appear to have subclinical liver disease (Reinhold, 1955).

Detection of residual liver damage, "recovery" stages of hepatitis, chronic passive congestion, portal cirrhosis: bromsulfalein, direct and total bilirubin, flocculation tests, serum albumin and globulin, or electrophoretic protein pattern, prothrombin, serum cholinesterase, urine urobilinogen and coproporphyrin [*see* Kunkel *et al.* (1947)].

Evaluation of the course of the jaundiced patient suffering from parenchymatous liver disease: serum direct and total bilirubin, flocculation tests, serum albumin and globulin, prothrombin. In addition, if severe, serum esterified cholesterol or cholinesterase. Blood urea N or NPN, glucose, and serum electrolytes also are important [*see* Kimmel, *et al.* (1954)].

Jones (1955) mentions an abrupt or steady rise in serum bilirubin, and declining serum albumin, cholinesterase, and prothrombin, together with decreasing urine output and rise in blood leukocyte count as evidence of deteriorating liver function.

Differentiation of jaundice due to biliary disease from that due to parenchymatous disease: serum alkaline phosphatase, cephalin-cholesterol flocculation, and thymol turbidity, serum mucoprotein, electrophoretic protein pattern, prothrombin response, repeated fecal urobilinogen tests.

Among the recent papers that discuss the differential diagnosis of jaundice are those of Mellinkoff *et al.* (1952), Popper and Schaffner (1952), Ricketts (1953), and Wang (1953).

Differentiation of extrahepatic biliary obstruction due to calculus from that due to neoplasm, stricture, etc.: fecal urobilinogen.

For following the course of the surgical patient with disease of the biliary tract: plasma prothrombin, phosphatase, serum direct and total bilirubin, albumin and globulin, electrolytes, blood urea N or NPN, and serum lipids.

Differentiation of hemolytic jaundice: serum direct and total bilirubin, fecal urobilinogen, erythrocyte fragility, reticulocyte count.

A decision regarding the number of tests to be used requires experience and judgment. The information gained increases as the number of tests increases but with rapidly diminishing returns beyond an optimum that will vary in different patients. Usually, the tests listed will suffice for an initial study, with additional requests to be made if further information is needed. To apply simultaneously the entire group of tests listed in any of the categories would seldom be justified.

Comparatively little has been done to evaluate the advantage gained by the use of two or more tests in combination. Gutman and Hanger (1941) found that serum phosphatase and cephalin-cholesterol flocculation supplemented each other for the diagnosis of common duct disease and that combining the findings of the two tests substantially improved the accuracy. Maclagan (1947) compared a number of tests singly and in pairs. Thymol turbidity alone gave correct diagnoses in 22 per cent of cases of liver disease that could be unequivocally classified as either obstruction or parenchymal. Serum alkaline phosphatase was correct in 46 per cent. However, a combination of thymol turbidity and alkaline phosphatase gave correct diagnoses in 79 per cent.

Wootton, King, and Maclean-Smith (1951) cite a statistical analysis by R. A. Fisher in which he used the method of discriminant functions to compare the effectiveness of four tests applied to jaundiced patients with that of two tests. The four-test group yielded only slightly more reliable information than a properly selected pair of tests. It is possible, therefore, to eliminate some procedures.

The practice in clinics where interest is centered on the study of liver disease, varies with respect to selection of the flocculation tests that are regularly used. Reliance on a single test is infrequent. Usually two or three tests are used. Probably the most frequent combination is the thymol test (Maclagan, 1944) and cephalin-cholesterol flocculation (Hanger, 1939). Often the zinc turbidity (Kunkel, 1947) or the ammonium sulfate turbidity (Huerga and Popper, 1949) are included. The colloidal red test (Ducci, 1947) gives results resembling closely those of the colloidal gold test applied to serum. The latter, according to Maclagan (1951), largely duplicates the results of simpler tests and thus no longer performs a useful service. Neefe *et al.* (1950) found no need for routine use of the colloidal red test in the study of viral hepatitis. A few clinics routinely use five or six tests, but this is done largely for the purpose of comparison and evaluation of the tests.

A comparison of a group of tests with respect to their behavior during the onset of viral hepatitis induced in volunteer subjects has been made

by Neefe and Reinhold (1946). Seven tests were abnormal in 95 per cent or more of the patients. These were the 1-minute direct bilirubin, urine bilirubin by the Harrison spot method, cephalin-cholesterol flocculation, thymol turbidity, thymol flocculation, colloidal gold, and bromsulfalein retention. Thus any of the seven might have served. However, the study showed that these tests differed distinctly in the times at which they first became positive. The bromsulfalein test showed a striking advantage in this respect and urine bilirubin ranked second. Had the reliance been placed only upon the thymol test, the hepatitis of some of the individuals might have escaped detection.

The ranking of the same group of tests in the same subjects was distinctly different when they were evaluated according to their ability to disclose persistence of the disease or its sequelae. In general the order is now reversed with the tests depending on changes in serum proteins showing a greater tendency to persist than those based on excretory function. Again dependence on a single test of either group would have meant failure to detect change in some patients. Furthermore, bromsulfalein retention persisted in some patients long after flocculation tests had become normal, so that dependence on the latter alone may fail to demonstrate liver damage in these circumstances. It appears inadvisable, therefore, to curtail too severely the number of procedures used, particularly when a wide variety of clinical material is being studied.

Quite frequently patients are encountered with equivocal signs of involvement of the liver which cannot be conclusively demonstrated by either clinical examination or laboratory studies. In such circumstances biopsy of the liver done either in the course of surgical exploration or by needle is considered essential. Besides providing helpful information about such patients, the widespread use of liver biopsy has greatly increased the understanding of liver disease. It has also enabled correlations to be made between the results of laboratory studies and the appearance of the liver under the microscope. A number of such studies have shown that there is a general correlation between the severity of the changes in the liver and the degree of change in the chemical tests used.

Popper (1951) found that the cephalin-cholesterol flocculation, thymol turbidity, and serum albumin and globulin concentrations showed a statistically significant correlation between degree of liver cell damage and degree of chemical abnormality. No significant correlation was found with phosphatase, prothrombin activity, urine urobilinogen, serum total cholesterol, or blood sedimentation rate.

Moyer and Wurl (1951) found the bromsulfalein test to be most consistently correlated. Schneider *et al.* (1953) report a good correlation in

portal cirrhosis between biopsy findings and serum-albumin concentration, cephalin-cholesterol flocculation, serum bilirubin, and bromsulfalein retention. Correlation with the thymol turbidity was poor.

At times the results of chemical studies will be within normal limits despite the existence of pathologic changes in the sample of liver tissue. Frequently, however, the results of the chemical studies will be abnormal in the absence of conclusive indications of abnormality in the liver sections (Christian, 1952).

There are a number of explanations for such discrepancies, among them the possibility of sampling errors in removing the 10 mg. (or less) sample of liver that a needle biopsy yields. It is probable also that methods of staining and examining sections of liver fail to demonstrate changes in cell function. The greatest utility of each method for the study of liver disease will be realized if liver biopsy and chemical studies are coordinated. In such a plan, chemical studies will serve for screening and evaluation of the patients and for following the clinical course. Liver biopsy will supplement such studies, particularly in patients who present diagnostic problems. The coordinated use of liver biopsy and biochemical studies is discussed by Popper and Schaffner (1952).

On rare occasions, chemical studies of patients with relatively substantial involvement of the liver may fail to demonstrate significant abnormalities (Ricketts, 1951). The use of chemical methods to study the clinical course also may have limitations. As an example, the patient studied by Flynn and Walshe (1951) before and after resection of about one sixth of the liver showed no change in serum bilirubin, prothrombin, bromsulfalein retention, hippuric-acid excretion, or alkaline-phosphatase activity. Some deterioration of the flocculation tests and an excessive output of urobilinogen in urine gave the only substantial evidence of altered status.

Another curious phenomenon, and one deserving more study, is the "hyperfunction" of the liver that may occur in mild forms of liver disease (Rosenberg and Soskin, 1941; Zieve and Hill, 1953).

Table 1 summarizes the prevailing response obtained when tests for the bile pigments and other procedures commonly used are applied in study of diseases of the liver parenchyma and the contrast in the response in disease of the biliary tract. Table 2 shows the response as reported in a variety of other diseases.

The causes of abnormal hepatic tests in these miscellaneous diseases are varied. Liver disease, antecedent to or not necessarily related to the disease diagnosed, may exist. Bogoch *et al.* (1955) found that 7 of 15 patients with diabetes mellitus had abnormal hepatic tests because of

Table 1. Prevailing Response of Hepatic Tests in Diseases of Liver and Biliary Tract

Syndrome	*Bilirubin and urobilinogen*	*Tests for parenchymal involvement*	*Tests for biliary-tract involvement*
Viral hepatitis without jaundice	May be present in urine and may increase slightly in serum. Urobilinogen may increase in urine.	BSF usually abnormal. CCF, TT, TF, ZT, one or more, may be abnormal.	Normal
Viral hepatitis with jaundice	Increased in serum and urine. Urobilinogen generally increased in urine and feces but may be absent.	Abnormal. Lowered mucoprotein.	Mainly normal but may be abnormal.
"Toxic" hepatitis: cholangiolitic jaundice	Increased in serum and urine.	CCF, TTT, TF, ZT may be normal or abnormal. A/G usually normal.	Increased serum phosphatase, lipid. Similar to extrahepatic obstruction.
Laennec's cirrhosis	May or may not be abnormal in serum and urine. Urobilinogen variable.	BSF abnormal. CCF, TT, TF, or ZT abnormal in about ¾. A/G abnormal.	Variable. Phosphatase may be high.
Extrahepatic obstruction			
Partial	Variable. Intermittent or continuous elevation.	Generally normal, but liver parenchyma may become injured and tests positive.	Variable but with elevated serum phosphatase and lipid prevalent. Good response to vitamin K.
Complete	Extreme elevation. Very low fecal urobilinogen.	Same as preceding.	Elevated serum phosphatase and lipid. Good response to vitamin K.
Biliary cirrhosis (Ahrens *et al.*, 1950; Ricketts *et al.*, 1950)	Bilirubin elevated. Urobilinogen variable.	BSF increased. A/G abnormal. TT increased. CCF increased about half.	Elevated phosphatase. Marked elevation of serum lipid, especially phospholipid.

BSF, bromsulfalein; CCF, cephalin-cholesterol flocculation; TF, thymol flocculation; TT, thymol turbidity; ZT, zinc turbidity; A, serum albumin; G, serum globulin.

liver disease not directly related to the diabetes. Abnormal turbidity and flocculation tests are easily explained by the occurrence in many diseases of changes in serum proteins, sufficiently like those occurring in liver disease to permit abnormal reactions to occur. Elevated alkaline-phospha-

Table 2. Prevailing Response of Hepatic Tests in Miscellaneous Diseases

Syndrome	*Bilirubin and urobilinogen*	*Tests for parenchymal involvement*	*Tests for biliary-tract involvement*
Hemolytic jaundice	Total serum bilirubin moderately elevated; 1-minute bilirubin normal or slightly increased. Urine bilirubin negative. Fecal urobilinogen increased urine urobilinogen often normal.	Seldom abnormal but may become so due to hypoxia or other complications.	Seldom abnormal. Pigment stones may cause biliary obstruction.
Amebic hepatitis (Heller *et al.*, 1953)	Bilirubin variable.	CCF abnormal. TT normal.	Normal
Amebic abscess (Brem, 1955)	..	Elevated TT in 2/8. Elevated BSF in 1/8. Lowered A in 3/8.	Elevated phosphatase in 1/8.
Amyloidosis, secondary (Wald, 1954)	..	Elevated CCF in 11/12. BSF > 10% in 8/23. Decreased cholesterol ester in 13/20.	..
Lupus erythematosus (Kofman *et al.*, 1955)	Bilirubin elevated in 27%.	Elevated CCF in 70%. Elevated TT in 100% Elevated BSF in 47%.	Elevated phosphatase 31%.
Metastatic carcinoma of the liver (Thomas and Zimmerman, 1952)	..	Elevated CCF in 15/17. Elevated TT in 8/17. Elevated BSF in all.	..
(Simons, 1954)	Elevated	Elevated CCF in 19/56. Elevated TT in 30/59 upwards.	Elevated phosphatase in all.
(Rhees *et al.*, 1954)	Nonicteric	..	Elevated phosphatase in 68%. Elevated mucroprotein.
(Molander *et al.*, 1954)	..	Lowered cholinesterase.	..
Hepatocellular adenoma (1 case) (Stumpf and Liber, 1954)	Normal	CCF and TT elevated. BSF 32%.	Phosphatase markedly elevated.
Multiple myeloma (Walsh *et al.*, 1955)	..	Elevated CCF in 36%. Elevated TT in 15%.	..

(table continued on page 410)

Table 2—*Continued*

Syndrome	*Bilirubin and urobilinogen*	*Tests for parenchymal involvement*	*Tests for biliary-tract involvement*
Chronic congestive heart failure (Evans *et al.*, 1952)	Bilirubin increased in 1/4.	Floc. and turbidity tests 18–41% positive. BSF elevated in severe failure.	..
Obesity (Zelman, 1952)	Elevated urine urobilinogen in 6/19. Bilirubin increased in 3/20.	Elevated BSF in all. CCF abnormal in 4/20. TT in 1 9/20.	Elevated phosphatase in 5/19.
Infectious mononucleosis (Brown *et al.*, 1949)	..	Elevated CCF in 38/39. Elevated TT in 19/39.	..
Rheumatoid arthritis (Darby, 1953)	..	Elevated BSF in 23%. Elevated flocculation tests in 73%.	..
Injury to spinal cord (Cooper *et al.*, 1951)	Bilirubin increased in 1/8.	Increased BSF in 6/8. Increased CCF in 1/8.	..
Weil's disease (Chinn *et al.*, 1951)	Bilirubin increased in 5/6. Urobilinogen in 4/4.	Increased CCF in 5/6. Increased TT in 3/6.	Moderately increased phosphatase in 5/6.
Diabetes mellitus (Bogoch *et al.*, 1955)	Bilirubin normal.	Increased BSF in 4/8. Increased G 6/8. Decreased albumin in 5/8.	Slightly increased alk. phosphatase in 1/8.

BSF, bromsulfalein; CCF, cephalin-cholesterol flocculation; TF, thymol flocculation; TT, thymol turbidity; ZT, zinc turbidity; A, serum albumin; G, serum globulin.

tase activities may be a result of bone involvement—for example, osteitis deformans, a fairly frequent illness of older people. Some of the abnormalities in liver function reported in miscellaneous illness are the result of inadequately defined standards of normal. Insufficient attention has been given to factors such as age, sex, race, and diurnal variations. Faulty technic also may contribute. The use of actual instead of ideal weight in calculating bromsulfalein dosages is an example. Erroneously high retention may occur in adipose subjects. When all these possibilities are excluded, it remains well established that certain diseases are accompanied by significant involvement of the liver.

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Erratum

On page 320 (Vol. 1, 1955), the second and third sentences in the last paragraph should read, "It is interesting that most of the values are quite low. Among the few high values is Number 8, a man about 45 years old who proved to be an alcoholic and may have had some liver dysfunction."

MINUTES OF THE MEETING OF THE AACC EXECUTIVE COMMITTEE

The National Executive Committee of the American Association of Clinical Chemists met in Minneapolis, Minn., on September 14, 1955. Those present were: Otto Schales (President), Max M. Friedman (National Secretary), Louis B. Dotti (National Treasurer), Monroe E. Freeman, Joseph H. Gast, Samuel Natelson, Margaret M. Kaser (Alternate for Icie M. Hoobler), Joseph I. Routh (Alternate for William G. Goldwater), Hugh J. McDonald (by invitation), Albert E. Sobel (by invitation), and David Seligson (by invitation).

Appointments

Committees of the Association were continued, with the following new appointments:

Program Committee: Joseph H. Gast, Chairman.

Standard Methods: David Seligson, Chairman; Joseph H. Gast, Margaret M. Kaser, Miriam Reiner, John G. Reinhold, and Joseph I. Routh.

Clinical Chemistry

Harold D. Appleton submitted a statement on behalf of the Board of Editors of CLINICAL CHEMISTRY. Manuscripts are being received in such quality and quantity that there is no problem in meeting the present page allotments. Thought is already being given to the expansion of the present journal. Advertising support is encouraging, and as of September 1, 1955, subscriptions have exceeded 2100.

Louis B. Dotti submitted a Treasurer's report showing a substantial increase in assets since one year ago. The report which follows does not include royalties for 1955 accruing from CLINICAL CHEMISTRY. The items for the Association's former publication, *The Clinical Chemist* refer to the last three issues in 1954.

Treasurer's Report

JULY 1, 1954 TO JUNE 30, 1955

Income

Membership dues	$6602.12
Royalties from Academic Press	303.42
Bank interest	71.04
Directory	90.00
The Clinical Chemist	350.42
Membership certificates	32.00
Subscriptions to CLINICAL CHEMISTRY	15.20
TOTAL	$7464.20

Expenses

CLINICAL CHEMISTRY subscriptions	$3600.00
CLINICAL CHEMISTRY	125.75
The Clinical Chemist	1399.70
Section allotments	257.60
Certificates	5.10
Directory	166.86
National Secretary	389.96
National Treasurer	106.40
Membership Committee	91.85

Subscriptions to Clinical Chemistry		15.40
Refund to members		67.60
Total		$6226.22
Total income	$7464.20	
Total expenses	6226.22	
Balance	$1237.98	
Bank balance July 1, 1954	2518.13	
Bank balance July 1, 1955	$3756.11	

Ernst Bischoff Award

The Executive Committee recommended that nominations for the Ernst Bischoff Award be expedited by requiring the nominator to give only a brief resume of the nominee, including only his important contributions. It was felt that heretofore members refrained from submitting nominations because of the amount of time required. It was also recommended that a nomination may be renewed for a following year merely upon the request of the nominator.

International Participation

Monroe E. Freeman, the Association's representative to the Commission on Clinical Chemistry and the International Federation of Clinical Chemistry, reported on the Commission meetings in Zurich, Switzerland, July 21–27, 1955.

Albert E. Sobel discussed the plans for the International Congress on Clinical Chemistry to be held in New York City during the week of September 9, 1956, under the auspices of the American Association of Clinical Chemists. Approval was given for making available the sum of $3000.00 for expenses incidental to the International Congress, these funds to be released at the discretion of the National Treasurer. It was moved, seconded, and passed that Sobel be authorized to appoint all the necessary committees for the International Congress, subject to approval by the President of the Association.

Standard Methods

David Seligson reported for the Editorial Committee of *Standard Methods*. This committee had met the previous evening and submitted a prospectus for Volume II. After detailed discussion of the form that *Standard Methods* should take, the Executive Committee voted that the series be composed of individual volumes, rather than continuous revised editions.

David Seligson also reported on the project of standard samples, to be carried out by the same editorial committee. It was proposed that a study be made to establish standard samples for certified constituents, this certification to be made by the American Association of Clinical Chemists. It was further proposed that secondary standards be established for such methods as required turbidity measurements. This study would assemble data for statistical variations and "normal" ranges and correlate them with *Standard Methods*. Various sources of financial support were indicated.

Investment Program and Budget Committee

It was moved, seconded, and approved that the Association purchase

a $500.00 savings bond as a start for an investment program. It was also voted that the National Treasurer be bonded for $10,000, to conform with legal requirements.

It was moved, seconded, and passed that a budget committee be appointed, this committee to approve all expenditures not previously allocated by the Executive Committee. This budget committee will consist of Louis B. Dotti, Max M. Friedman, and one additional member yet to be designated.

Membership and Dues

It was moved, seconded, and passed that the Executive Committee recommend to the membership that dues for 1956 be kept at $11.00 for members and $7.50 for associate members. (This was subsequently voted at the membership meeting held on September 15, 1955.)

Since the dues of associate members are below the subscription rates of the journal they receive, and furthermore part of those dues is returned as section allotments, it was moved, seconded, and passed that beginning with January 1, 1958, those associate members who will have been at that time associate members for a period of 4 years shall be assessed full member dues. After that date *newly elected* associate members shall be permitted 4 years at the reduced dues. Assessment to full member dues shall not be construed as promotion to full membership.

MAX M. FRIEDMAN
National Secretary

SEVENTH ANNUAL MEETINGS

The annual meetings of the American Association of Clinical Chemists in Minneapolis were held September 12–16, 1955, in conjunction with the meetings of the American Chemical Society. The program and accounts of the sessions have been detailed in recent issues of *Chemical and Engineering News*.

There were 195 papers given in the Division of Biological Chemistry, most of which were of interest also to clinical chemists. Also included were two sessions devoted to clinical chemistry and a symposium on "Newer Techniques Applicable in Clinical Chemistry."

The social highlight, as usual, was the annual dinner at which Otto Schales, President of the AACC, presented the Ernst Bischoff Award.

The exact attendance at the seventh annual meetings is not known, since registration was through the American Chemical Society, but the large numbers who attended the sessions on clinical chemistry attest to the increasing interest in this chemical specialty.

ERNST BISCHOFF AWARD

The fourth Ernst Bischoff Award was presented at the annual dinner of the Association in Minneapolis, Minn., on September 15, 1955. The recipient was Michael X. Sullivan, Professor of Biochemistry at Georgetown University, Washington, D. C. The ceremonies were presided over by Otto Schales, President of the AACC, and

Sullivan was cited "for his contributions to clinical chemistry as the author of numerous analytical methods in the field of biochemistry, for his devoted efforts in the instruction and education of biochemists, and for his contributions to the progress of the knowledge of proteins and especially of sulfur compounds."

Doctor Sullivan accepted the Award with an address on "Highly Specific Tests in Clinical Chemistry." This was enthusiastically received by an audience that included several of his former students.

The Ernst Bischoff Award is given annually by the American Association of Clinical Chemists to a scientist for his outstanding contributions to clinical chemistry. The Award consists of a scroll, a medal, and an honorarium of $500.00. The recipient is selected by the Honorary Members of the Association from a list of nominees submitted by the membership at large.

Members are urged to forward nominations as soon as possible for the 1956 Ernst Bischoff Award, which will be presented during the International Congress in New York City to be held during the week of September 9, 1956. The brief should be sent in triplicate, typewritten, to Professor Joseph H. Roe, George Washington University, School of Medicine, Washington, D. C.

ASSOCIATE MEMBERS

The minutes of the Executive Committee meeting in Minneapolis includes a change of the dues status of associate members, to begin on January 1, 1958. The dues for that membership category are now less than the subscription to the Association's journal, which all members receive, and part of the dues is returned to the local section as section allotments.

After January 1, 1958, an associate member will be permitted 4 years of reduced dues, following which the dues will be the same as for full members. At that date this regulation will become retroactive for all members.

Increase of dues to full membership rates shall not be construed as promotion to full membership. An associate member may at any time request upgrading by writing to the Committee on Membership to that effect and submitting, in justification of such a request, data additional to those concerning the original application. The national office does not have the facilities for automatic periodic review of the status of all members as has been suggested. It therefore becomes the responsibility of each associate member individually to request such a review.

Any associate member desiring such a change in status should address a communication to that effect to Dr. Harry Sobotka, The Mount Sinai Hospital, New York 29, N. Y. A list of requirements for full membership will be sent on request.

INTERNATIONAL CONGRESS ON CLINICAL CHEMISTRY

Plans are now moving ahead in preparation for the International Congress on Clinical Chemistry to be held in New York City during the week of September 9, 1956. A large

and enthusiastic audience is anticipated. This will be the first opportunity for clinical chemists from abroad to visit the United States during a formal conclave on clinical chemistry. There will be the added advantage in that the American Chemical Society meetings are to be held in Atlantic City during the week of September 16, 1956. This will permit attendance at both meetings with little additional travel expense.

At this writing it is not yet possible to give the details of the program for the International Congress. There will, however, be several days of scientific sessions, and chemists are advised to submit abstracts as soon as possible. Symposia are being organized by invitation. A social and ladies' program will of course be included.

NEWS FROM THE SECTIONS

Chicago Section

The present officers of the Chicago Section are as follows:

Chairman: A. Dubin (Director of Chemistry, Hektoen Institute, Cook County Hospital)

Vice-Chairman: G. S. Stewart (Department of Biochemistry, Presbyterian Hospital)

Secretary: H. Feinberg (Cardiovascular Department, Medical Research Institute, Michael Reese Hospital)

Treasurer: A. Kaplan (Department of Biochemistry, Medical Research Institute, Michael Reese Hospital)

Executive Committee at Large:

C. Chen (Department of Biochemistry, Northwestern Medical School)

H. J. McDonald (Department of Biochemistry, Stritch School of Medicine, Loyola University)

H. F. Weisberg (Department of Biochemistry, Mt. Sinai Hospital)

Midwest Section

As prescribed by Section By-Laws, an election of officers was held in the Midwest Section at the last meeting of the spring session. The new officers for the coming year are:

Chairman: Robert L. Dryer

Vice Chairman and Program Director: Kurt M. Dubowski

Secretary-Treasurer: Robert S. Melville

Throughout this past year the Section held meetings at various locations, including the Mayo Clinic in Rochester, Minn., the State Univesity of Iowa in Iowa City, and the Medical Associates Clinic in Dubuque, Iowa.

Of outstanding interest was the first Annual Instrument Clinic and demonstration held at the State University of Iowa on March 31 and April 1. A wide variety of instruments and equipment of optical, physiological, microchemical, radioisotopic, and general analytical interest was displayed.

Plans are being made for even more extensive clinics to be held in the future. The purpose of these clinics is to acquaint workers in various fields with available instruments and to give them an opportunity to discuss their problems with the appropriate technical representatives of the instrument industry.

The American Association of Clinical Chemists

Program of the Annual Meeting

To Be Held in Conjunction with the National Meetings of the American Association for the Advancement of Science, Atlanta, Georgia

December 26, 1955, 2 p.m., Dixie Ballroom, Henry Grady Hotel

Symposium: Recent Concepts in Clinical Chemistry

ALBERT E. SOBEL, *Presiding*

1. Opening Remarks. ALBERT E. SOBEL, The Jewish Hospital of Brooklyn, Brooklyn, N. Y.
2. Role of the Clinical Chemist in the Hospital. JOSEPH H. GAST, Baylor University, College of Medicine, Houston, Texas
3. New Concepts in Acid-Base Balance. SAMUEL NATELSON, Rockford Memorial Hospital, Rockford, Ill.
4. Studies on Lipid Metabolism. D. B. ZILVERSMIT, Department of Physiology, University of Tennessee, Memphis, Tenn.
5. Blood Proteins. HENRY G. KUNKEL, Rockefeller Institute for Medical Research, New York, N. Y.

AACC Dinner

7 p.m., Dixie Ballroom, Henry Grady Hotel

December 27, 1955, 9 a.m., Room B, Henry Grady Hotel

Analytical Methods

I. J. GREENBLATT, *Presiding*

1. The Use of Infrared Spectroscopy in Clinical Chemistry. DIETRICH E. BEISCHER, U.S. Naval School of Aviation Medicine, Pensacola, Fla.
2. Cyanomethemoglobin "Certified Hemoglobinometry Standards" as a Reference in Other Spectrophotometric Procedures. EUGENE W. RICE, Clinical Laboratories, Presbyterian and Women's Hospitals, and Department of Pathology, School of Medicine, University of Pittsburgh, Pittsburgh, Pa.
3. An Ultra Micro Method for the Determination of Proteins in Biological Fluids. GEORGE R. KINGSLEY and GLORIA GETCHELL, Department of Physiological Chemistry, School of Medicine, University of California at Los Angeles, and the Clinical Biochemistry Laboratory, Veterans Administration Center, Los Angeles, California.
4. The Effect of High Salt Concentration on Color Production of the Protein-Biuret Reaction. HAROLD L. ROSENTHAL and TOYOKO

KAWAKAMI, Division of Chemistry, Clinical and Pathological Laboratories, Rochester General Hospital, Rochester, N. Y.

5. A Simplified and Safer Urea Determination. AARON S. GOLDBERG, Department of Chemistry, Bronx Hospital, New York, N. Y.
6. A Simplified Device for the Estimation of Urea. R. L. DRYER, J. I. ROUTH, and A. R. TAMMES, Clinical Biochemistry Laboratory, State University of Iowa, Iowa City, Iowa.
7. The Distribution of C^{14}-Labeled Salicylic Acids in Animal Tissues. R. W. KNOUSE, J. I. ROUTH, and W. D. PAUL, Clinical Biochemistry Laboratory, State University of Iowa, Iowa City, Iowa.

December 27, 1955, 2 p.m., Room B, Henry Grady Hotel

Metabolism and Analytical Methods

SAMUEL NATELSON, *Presiding*

8. The Determination of Oxalate in Urine. ESTELLE R. HAUSMAN, JOHN S. MCANALLY, and GEORGE T. LEWIS, Department of Biochemistry, School of Medicine, University of Miami, Coral Gables, Fla.
9. Glycogenesis in the Liver Following Parenteral Administration of Fructose, Glucose, and Invert Sugar. JOSEPH H. ROE, ROBERT W. LONGLEY, and OLAF ALVIG, Department of Biochemistry, School of Medicine, George Washington University, Washington, D. C.
10. Elevation of Rabbit Blood Glycogen Following Parenteral Prednisone. I. J. GREENBLATT, A. S. WATNICK, R. WAYNE, and H. ALBAUM, Beth-El Hospital, and Brooklyn College, Brooklyn, N. Y.
11. Vitamin A Absorption in Mongolism. ALBERT E. SOBEL, M. STRAZZULIA, B. S. SHERMAN, B. ELKAN, S. W. MORGENSTERN, N. MARIUS, and A. MEISEL, Departments of Biochemistry and Pediatrics, The Jewish Hospital of Brooklyn, Brooklyn, N. Y.
12. Serum Iron in Liver Disease. EMANUEL E. MANDEL and LEONA NIESPODZANY, The Chicago Medical School, Chicago, Ill., and the Communicable Disease Center, U. S. Public Health Service, Atlanta, Ga.
13. The System of Analysis on the Essential Blood Electrolytes and Distribution of Sodium and Potassium in Plasma and Erythrocytes. V. M. SYCHEFF, The French Hospital Clinical Laboratory, San Francisco, Calif.
14. A Comparative Study of the Estimation of Vitamin B_{12}. H. BAKER, H. SOBOTKA, I. PASHER, and S. H. HUTNER, The Mount Sinai Hospital, Department of Chemistry, and Haskins Laboratory, New York, N. Y.

Index to Volume 1

Abnormalities in blood clotting, in liver disease, 379
Absorption, fat, 98
Adlersberg, David: Electrophoresis and Monomolecular Layer Studies with Serum Lipoproteins, 18
Akerson, Irving B., see Gottfried, S. P.
Albumin, serum, denaturation of, 181
American Board of Clinical Chemistry, 137, 277
Amylase: Turbidimetric method, 159
A New Journal: Appleton, H. D., 1
A Note on Quantitative Urobilinogen Determinations: Balikov, B., 264
Anderson, J. T., see Keys, A.
Appleton, Harold D.: A New Journal, 1
A Practical Manual of Medical and Biological Staining Techniques: Gurr, E. (Book Review), 81
Arginase: Blood, method, 110
Balikov, Bernard: A Note on Quantitative Urobilinogen Determinations, 264
Becker, E., see Friedman, M. M.
Beer's Law: for stained protein patterns on filter paper, 329
Beher, W. T., see Gaebler, O. H.
Berger, Eugene Y.: Calcium Determination in Biologic Material, 249
Bile-pigment metabolism, 391
Bischoff, Ernst, Award, 72, 346, 423, 424
Bluemle, Dolores B., see Singer, R. B.
Bossak, E. T., see Adlersberg, D.
Brown, Herman, see Samson, M.
Brown, Ray K.: Serum Lipoproteins, Chemical and Enzymatic Studies, 83
Butt, Arthur J., see Leonard, R. H.
Calcium: EDTA method, 125: cerate oxidimetry method, 250
Calcium Determination in Biologic Material: Berger, E. Y., 249
Calculi, Quantitative method for urinary, 241
Cannan, R. Keith: Proposal for the Distribution of a Certified Standard for Use in Hemoglobinometry, 151
Carbohydrate metabolism in liver disease, 359
Carbon Monoxide, blood, micro-method, 170
Cell Volume, Blood, micro-method, 299
Cephalin-Cholesterol Flocculation Test, 377
Chemical Abnormalities in Blood Serum Associated with the Carrier State of Viral Hepatitis: Reinhold, J. G., 3
Chemical Evaluation of the Functions of the Liver, (Revised): Reinhold, J. G., 351
Chemical Physiology of Liver Disease, 358
Chemotherapy of Infections: Collier, H. O. J., (Book Review), 81
Cholesterol: Determination with p-toluenesulfonic acid, 345: Effect of Diet on blood, 34
Clinical Chemistry in Practical Medicine: Stewart, C. P. and Dunlop, D. M., (Book Review), 80
Clinical Significance of the Magnesium: Calcium Ratio; Technic for the determination of Magnesium and Calcium in Biologic Fluids: Friedman, H. S. and Rubin, M. A., 125
Coco, S., see Mason, W. B.
CO_2 Content, micro-determination, 293
Code of Ethics of the American Association of Clinical Chemists, 203
Colorimetric Determination of Blood Arginase: Friedman, M. M. and Becker, E., 110
Convenient Apparatus for the Continuous Dehydration of Histologic Material: Nadeau, G. and Joly, L. P., 190
Cooper, Gerald E., see Mandel, E. E.
Copper and diseases of the liver, 387
Cross-Contaminator Exclusion Test for Dry-Ashing Determination of Protein-Bound Iodine: Samson, M., Brown, H. and Eichen, S., 269
De Lalla, L. S., see Brown, R. K.
Detoxification reactions of the liver, 387
Digestion, fat, 93
Di Mauro, S., see Gottfried, S. P.
Diseases of the liver and biliary tract, 354
Disturbances of water and electrolyte balances in liver diseases, 384
Effect of Shaking on the Accuracy of Salt

Fractionation Methods for Serum Albumin: Saifer, A. and Zymaris, M. C., 180
Effects of Diet on Blood Lipids in Man: Keys, A., Anderson, J. T., Fidanza, F., Keys, M. H. and Swahn, B., 34
Eichen, S., see Samson, S.
Electrometric pH Determinations: Theory and Practice: Bates, R. G. (Book Review), 149
Electrophoresis and Monomolecular Layer Studies with Serum Lipoproteins: Adlersberg, D., Bossak, E. T., Sher, I. H. and Sobotka, H., 18
Estimation of Hydrocortisone Secretion: Silber, R. H., 234
Estimation of Lipid Fractions of Blood Plasma; System Using the Allen Volumetric Method: Kibrick, A. C. and Skupp, S. J., 317
Fatty Acids: Blood, Iodine numbers, 324: Blood plasma, 320: Blood, thiocyanogen numbers, 324
Fidanza, F., see Keys, A.
Freeman, Monroe E: President's Address, 68
Friedman, Howard S.: Clinical Significance of the Magnesium: Calcium Ratio, 125
Friedman, Max, M.: Colorimetric Determination of Blood Arginase, 110
Friedman, Nathan, H., see Gottfried, S. P.
Gaebler, O. H.: Reproducibility and Validity of Sodium Sulfate Fractionation of Proteins in Plasma and Knee Joint Fluid, 105
Galpin, R. R., see Gaebler, O. H.
Gordon, B. S., see Klein, B.
Gorsuch, T. L., see Mandel, E. E.
Gottfried, Sidney P.: Serum Beta-Lipoproteins of Normal, Atherosclerotic and Lipemic Individuals, 253
Graef, I., see Klein, B.
Green, D. E.: Oxidation and Synthesis of Fatty Acids in Soluble Enzyme Systems of Animal Tissues, 53
Hemochromatosis: 118
Hemoglobin: cyanmethemoglobin standard, 153: method, 153
Hepatic tests: evaluation of, 8: on blood donors, 10
Hepatitis: 3
Hormone metabolism in liver disease, 382
Human Biochemistry: Kleiner, I. S., (Book Review), 82
Hydrocortisone: method for calculation of secretion, 235
Iodine and Thiocyanogen Numbers of the Fatty Acids of Blood Plasma: Kibrick, A. C. and Skupp, S. J., 324
Improved Methods of Analysis for Oxygen, Carbon Monoxide and Iron on Fingertip Blood: Natelson, S. and Menning, C. M., 165
Iron: Blood, micro-method, 172: tissues, 118
Iron Content of Tissues in Exogenous and Endogenous Hemochromatosis: Klein, B., Gordon, B. S. and Graef, I., 118
Joly, L. P., see Nadeau, G.
Jones, Betty Jo: p-Toluenesulfonic Acid, Cholesterol Determination, and Explosions, 345
Kauffman, D. L., see Brown, R. K.
Keys, Ancel: Effect of Diet on Blood Lipids In Man; particularly Cholesterol and Lipoproteins, 34
Keys, M. H., see Keys, A.
Kibrick, Andre C.: Estimation of Lipid Fractions of Blood Plasma, 317
Kibrick, Andre C.: Iodine and Thiocyanogen Numbers of the Fatty Acids of Blood Plasma, 324
Klein, Bernard: Iron Content of Tissues in Exogenous and Endogenous Hemochromatosis, 118
Laboratory Instructions in Biochemistry: Kleiner, I. S. and Dotti, L. B., (Book Review), 82
Laurence, D. J. R., see Rees, V. H.
Legal Medicine, Pathology and Toxicology: Gonzales, T. A., Vance, M., Helpern, M. and Umberger, C. J., (Book Review), 148
Legislation, Clinical Chemistry, 347
Leonard, Reid H.: Quantitative Identification of Urinary Calculi, 241
Lipids: Fractions in blood plasma, method, 318: in liver disease, 360
Lipids, The: Their Chemistry and Biochemistry: Deuel, H. J., Jr. (Book Review), 219
Lipoproteins: 18, alteration by enzymes, 86: beta, changes at 6.5° and 37.5°, 255: effect of diet, 34: in human disease, 26: in mammals, 22: preparation, 84: Monomolecular layer studies, 18
Magnesium: method for biologic fluids, 125

Mandel, Emanuel E.: Seromucoid in Hepatobiliary Disease, 221
Mason, W. Burkett: Purification of Chromium (VI) for Use in the PBI Determination, 117
Menning, C. M., see Natelson, S.
Monomolecular Layer Studies: Lipoproteins, 18
Moreland, Ferrin B., see Jones, B. J.
Nadeau, Guy: Convenient Apparatus for the Continuous Dehydration of Histologic Material, 190
Natelson, Samuel: Improved Methods of Analysis for Oxygen, Carbon Monoxide and Iron on Fingertip Blood, 167
Neutral Fats: plasma, 320
Nitrogen metabolism in liver disease, 364
Organic Analysis: Mitchell, J. Jr., Kolthoff, I. M., Proskauer, E. S., and Weissberger, A., (Book Review), 148
Oxidation and Synthesis of Fatty Acids in Soluble Enzyme Systems of Animal Tissues: Green, D. E., 53
Oxygen: blood micro-method for capacity and content, 166
Peralta, Oscar: Rapid Estimation of Amylase Activity of Serum by Turbidimetry, 157
pH: Blood micro-colorimetric determination, 288
Plasma proteins in liver disease, 368
Pope, R. H., see Gottfried, S. P.
Practical Clinical Biochemistry: Varley, Harold, (Book Review), 218
President's Address: Freeman, M. E., 68
Proposal for the Distribution of a Certified Standard for Use in Hemoglobinometry: Cannan, R. K., 151
Protein Bound Iodine, 117, 269
Proteins: plasma, 105: Knee joint fluid, 105
p-Toluenesulfonic Acid, Cholesterol Determinations and Explosions: Jones, B. J. and Moreland, F. B., 345
Purification of Chromium (VI) for use in the PBI Determination: Mason, W. B. and Coco, S., 117
Quantitative Identification of Urinary Calculi: Leonard, R. H. and Butt, A. J., 241
Rapid Estimation of Amylase Activity of Serum by Turbidimetry: Peralta, O. and Reinhold, J. G., 157
Recent Studies of Fat Digestion and Absorption: Reiser, R., 93
Recognition of Specialists in Other Fields: Turner, E. L., 196
Rees, V. H.: The Correspondence with Beer's Law for the Optical Density of Stained Protein Patterns on Filter Paper as a Function of Surface Protein Concentration, 329
Reiser, R., Recent Studies of Fat Digestion and Absorption, 93
Reinhold, John G.: Chemical Abnormalities in Blood Serum Associated with the Carrier State of Viral Hepatitis, 3
Reinhold, John G.: Chemical Evaluation of the Functions of the Liver (Revised), 351
Reinhold, John G., see Peralta, O.
Reproducibility and Validity of Sodium Sulfate Fractionation of Proteins in Plasma and Knee Joint Fluid: Gaebler, O. H., Beher, W. T., Sigler, J. W. and Galpin, R. R., 105
Rubin, M. A., see Friedman, H. S.
Saifer, Abraham: Effect of Shaking on the Accuracy of Salt Fractionation Methods for Serum Albumin, 180
Samson, Meyer: Cross-Contaminator Exclusion Test for Dry-Ashing Determination of Protein Bound Iodine, 269
Selection of procedures in liver disease, 403
Seromucoid in Hepatobiliary Disease: Mandel, E. E., Gorsuch, T. L. and Cooper, G. E., 221
Serum alkaline phosphatase, 390
Serum Beta-Lipoproteins of Normal, Atherosclerotic and Lipemic Individuals: Gottfried, S. P., Pope, R. H., Friedman, N. H., Akerson, I. B., and Di Mauro, S., 253
Serum iron in liver disease, 385
Serum Lipoproteins; Chemical and Enzymatic Studies: Brown, R. K., De Lalla, L. S. and Kauffman, D. L., 83
Sher, I. H., see Adlersberg, D.
Shohl, Jane, see Singer, R. B.
Sigler, J. W., see Gaebler, O. H.
Silber, Robert H.: Estimation of Hydrocortisone Secretion, 234
Simultaneous Determination of pH, CO_2 Content and Cell Volume in 0.1 ml. Aliquots of Cutaneous Blood; A Modification of the Shock and Hastings Technic:

Singer, R. B., Shohl, J. and Bluemle, D. B., 287
Singer, Richard G.: Simultaneous Determination of pH, CO_2 Content and Cell Volume in 0.1 ml. Aliquots of Cutaneous Blood, 287
Skupp, S. J., see Kibrick, A. C.
Sobotka, H., see Adlersberg, D.
Statistical Analysis in Chemistry and the Chemical Industry: Bennett, C. A., and Franklin, N. L., (Book Review), 81
Structure of the liver, 351
Sullivan, Michael X.: 1955 Ernst Bischoff Award, 346
Swahn, B., see Keys A.
The Correspondence with Beer's Law for the Optical Density of Stained Protein Patterns on Filter Paper as a Function of Surface Protein Concentration: Rees, V. H. and Laurence, D. J. R., 329
Thiocyanogen Numbers of Plasma Fatty Acids, 324
Thymol Flocculation Test, 376
Thymol Turbidity Test, 373
Turner, Edward L.: Recognition of Specialists in Other Fields, 196
Urobilinogen: erratum for page 265, 349: quantitative method, 264
Zymaris, M. C., see Saifer, A.

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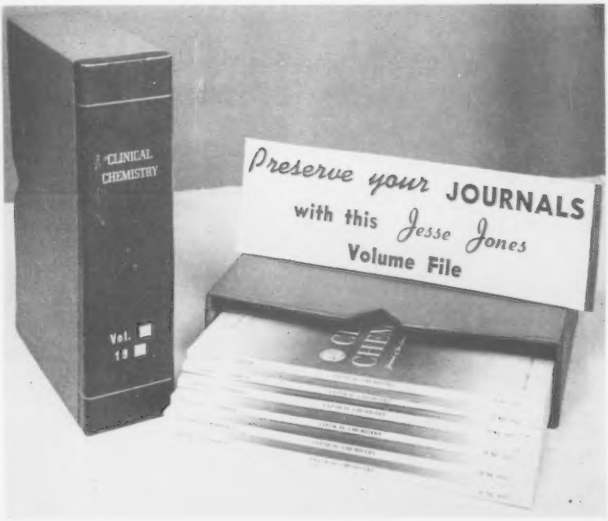

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